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2026

Document Version:
Publisher's PDF, also known as Version of record

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LUND UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on the 16th of January 2026 at 09.00 in Segerfalk Lecture Hall, BMC, Lund University, Lund, Sweden

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Organization: LUND UNIVERSITY

Department of Clinical Sciences Lund, the Section for Cardiology, Faculty of Medicine

Document name: Doctoral Dissertation **Date of issue:** January 16, 2026

Author: Salaheldin Ahmed

Sponsoring organizations: Actelion Pharmaceuticals Sweden AB, ALF, GoRadCare AB, Janssen-Cilag AB, Nordic Infucare, Swedish Society of Pulmonary Hypertension (SveFPH), Helsingborg Hospital, and Skåne University Hospital Foundations.

Title and subtitle:

Plasma tyrosine kinase and tumour related biomarkers in pulmonary hypertension differentiation and heart failure, before and after heart transplantation – utilising Lund Cardio Pulmonary Registry

Abstract:

Left heart failure (HF) is a common syndrome, in which pulmonary hypertension (PH) is a frequent complication. Both conditions typically present with nonspecific symptoms, complicating clinical assessment. Differentiating HF with preserved ejection fraction and PH (HFpEF-PH) from pulmonary arterial hypertension (PAH) can be challenging, but is crucial to reduce diagnostic delays, morbidity, and mortality. Heart transplantation (HT) remains the ultimate HF treatment, but may often be complicated by asymptomatic deterioration until graft dysfunction reaches fatal stages. The pathobiology of these conditions remains incompletely understood, but pathways involving tyrosine kinase and tumour signalling are increasingly recognised in both HF and PH.

Paper I and **II** investigated the relative plasma dynamics of tyrosine kinases and tumour-related proteins in relation to haemodynamics in advanced HF, before and one year after HT. **Paper III** assessed absolute levels of vascular and inflammatory plasma proteins pre-HT and at multiple post-HT follow-ups. **Paper IV** examined tumour-related proteins for distinguishing HFpEF-PH from PAH, while **paper V** explored the prognostic value of these proteins in left HF with PH. All studies were based on the prospective Lund Cardio Pulmonary Registry (LCPR). Plasma proteins were quantified using proximity extension assays (**papers I, II, IV, V**) and multiplex sandwich immunoassays (**paper III**).

In **paper I** and **II**, the plasma levels of vascular endothelial growth factor (VEGF-D), human epidermal growth factor receptor 4, endocan, and brother of CDO were elevated in advanced HF. Their plasma levels decreased one year after HT, approaching those of healthy controls, and correlated with several haemodynamic parameters following HT. In **paper III**, the elevated absolute plasma concentrations of VEGF-D and soluble fms-like tyrosine kinase-1 decreased most prominently during the first four weeks after HT and continued to decline throughout the first year. They were also intraindividually associated with haemodynamic parameters of pulmonary congestion, pulmonary hypertension, and cardiac function before- and at four weeks, six months, and one year post-HT. In **paper IV**, the combination of plasma alpha-1-microglobulin/bikunin precursor, lipoprotein lipase, and glyoxalase I distinguished HFpEF-PH from PAH with the highest accuracy. In **paper V**, five proteins were initially linked to transplantation-free survival in left HF with PH, but none remained prognostic after adjustment for age, sex, atrial fibrillation and systemic hypertension.

The present thesis highlights circulating biomarkers in advanced HF, both before and after HT, and in the differentiation of HFpEF-PH from PAH. Further investigation of these proteins may enhance our pathophysiological understanding as well as facilitate HFpEF-PH diagnosis and haemodynamic surveillance in advanced HF, and after HT.

Key words: AMBP, BOC, HER4, LPL, monitoring biomarkers, PH-LHD, right heart catheterisation.

Classification system and/or index terms (if any) – Supplementary bibliographical information –

Language: English **Number of pages:** 109

ISSN and key title: 1652-8220

ISBN: 978-91-8021-800-9

Recipient's notes – Price – Security classification –

Plasma tyrosine kinase and tumour related biomarkers in pulmonary hypertension differentiation and heart failure, before and after heart transplantation

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The cover background and figure were designed by Abdulla and Salaheldin Ahmed and illustrated by Abdulla Ahmed

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Department of Clinical Sciences Lund, the Section for Cardiology

ISSN 1652-8220

ISBN 978-91-8021-800-9

Lund University, Faculty of Medicine Doctoral Dissertation Series 2026:2

Printed in Sweden by Media-Tryck, Lund University

Lund 2025

In the Name of Allah, the Most Gracious, the Most Merciful

﴿ يَرْفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُوا مِنكُمْ وَٱلَّذِينَ أُوتُوا ٱلْعِلْمَ دَرَجَٰتٍ وَٱللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴾

Qur'an, 58:9

Allah (God) will exalt those who believe among you, and those who have knowledge, to high ranks. Allah is Informed of what ye do.

Pickthall M. The Meaning Of The Glorious Koran

- An Explanatory Translation (1948)

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List of papers

The thesis is based on the following papers, which are referred to in the text by their respective Roman numerals:

I. **Ahmed S**, Ahmed A, Säleby J, Bouzina H, Lundgren J, Rådegran G. Elevated plasma tyrosine kinases VEGF-D and HER4 in heart failure patients decrease after heart transplantation in association with improved haemodynamics. *Heart Vessels.* 2020;35(6):786-99.

II. **Ahmed S**, Ahmed A, Bouzina H, Lundgren J, Rådegran G. Elevated plasma endocan and BOC in heart failure patients decrease after heart transplantation in association with improved hemodynamics. *Heart Vessels.* 2020;35(11):1614-28.

III. **Ahmed S**, Lundgren J, Ahmed A, Rådegran G. Plasma VEGF-D and sFLT-1 are potential biomarkers of haemodynamics and congestion in heart failure and following heart transplantation. *JHLT Open*, Volume 2, 100013 (2023).

IV. **Ahmed S**, Ahmed A, Rådegran G. Plasma tumour and metabolism related biomarkers AMBP, LPL and Glyoxalase I differentiate heart failure with preserved ejection fraction with pulmonary hypertension from pulmonary arterial hypertension (2021). *Int. J. Cardiol.* 2021;345:68-76.

V. **Ahmed S**, Ahmed A, Rådegran G. Data on plasma tumour and metabolism related proteins' potential in differentiation of HFpEF-PH from PAH and in prognosis of left heart failure patients with pulmonary hypertension. *Data Brief.* 2022;40:107747.

Letters

VI. **Ahmed S**, Ahmed A, Rådegran G. The Authors' Reply to the Letter "Plasma tumour and metabolism related biomarkers differentiate PAH from HFpEF-PH may improve long-term prognosis". Int. J. Cardiol. 2022;348:108.

VII. **Ahmed S**, Ahmed A, Rådegran G. Structured evaluation of unclear dyspnea–An attempt to shorten the diagnostic delay in pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension. *Pulm. Circ.* 2024;14:e12340.

Scientific contributions linked to, but not included in, the present doctoral dissertation. These are categorised and listed chronologically from the earliest to the most recent, using Arabic numerals:

Reviews:

1. **Ahmed S**, Ahmed A, Rådegran G. Structured evaluation of unclear dyspnea - of great importance to early identify patients with PAH and CTEPH and improve prognosis. *Läkartidningen*. 2022;119:21238.
2. **Ahmed S**, Ahmed A, Rådegran G. Circulating biomarkers in pulmonary arterial hypertension: State-of-the-art review and future directions. *JHLT Open*. 2024;6:100152.

Letters:

1. Ahmed A, **Ahmed S**, Rådegran G. Risk stratification in pulmonary arterial hypertension - implementation of an internet-based risk calculator to guide treatment. *Läkartidningen*. 2023; 120:1254-1257.
2. Ahmed A, **Ahmed S**, Rådegran G. Risk assessment in pulmonary arterial hypertension patients with multiple comorbidities and/or advanced age—where do we stand and what's next? *Pulm. Circ.* 2023; 13:e12314.
3. Ahmed A, **Ahmed S**, Rådegran G. Risk assessment in pulmonary arterial hypertension: A step towards clinical implementation based on the 2022 ESC/ERS pulmonary hypertension guidelines. *Pulm. Circ.* 2023; 13:e12253.

Original articles:

1. Säleby J, Bouzina H, **Ahmed S**, Lundgren J, Rådegran G. Plasma receptor tyrosine kinase RET in pulmonary arterial hypertension diagnosis and differentiation. *ERJ Open Research*. 2019;5(4):00037-2019.
2. Ahmed A, **Ahmed S**, Arvidsson M, Bouzina H, Lundgren J, Rådegran G. Prolargin and matrix metalloproteinase-2 in heart failure after heart transplantation and their association with haemodynamics. *ESC Heart Failure*. 2020;7(1):224-35.
3. Ahmed A, **Ahmed S**, Arvidsson M, Bouzina H, Lundgren J, Rådegran G. Elevated plasma sRAGE and IGFBP7 in heart failure decrease after heart transplantation in association with haemodynamics. *ESC Heart Failure*. 2020;7(5):2340-53.
4. Ahmed A, **Ahmed S**, Rådegran G. Plasma ADAMTS13 and von Willebrand Factor in diagnosis and prediction of prognosis in pulmonary arterial hypertension. *Pulm. Circ.* 2021; 11:1-15 20458940211041500.

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9. Ahmed A, Kania K, Abdul Rahim H, **Ahmed S**, Rådegran G. Adrenomedullin peptides and precursor levels in relation to haemodynamics and prognosis after heart transplantation. *ESC Heart Failure.* 2023; 10:2427-2437.

10. Arvidsson M, Ahmed A, Säleby J, **Ahmed S**, Hesselstrand R, Rådegran G. Plasma TRAIL and ANXA1 in diagnosis and prognostication of pulmonary arterial hypertension. *Pulm. Circ.* 2023; 13:e12269.

11. Engel Sällberg A, Helleberg S, **Ahmed S**, Ahmed A, Rådegran G. Plasma tumour necrosis factor-alpha-related proteins in prognosis of heart failure with pulmonary hypertension. *ESC Heart failure*. 10: 3582–3591.

12. Westöö C, Mutgan AC, van der Have O, Mead TJ, **Ahmed S**, Lampei E, Koch CD, Norvik C, Aspberg A, Bech M, Peruzzi N, Brunnström H, Kwapiszewska G, Rådegran G, Apte SS, Tran-Lundmark K. Localization, Proteolytic Processing, and Binding Partners of Versican Isoforms in Vascular Lesions of Pulmonary Arterial Hypertension. *J. Histochem. Cytochem*. 2025;73(3-4):129-145.

13. Kania K, Ahmed A, **Ahmed S**, Tran-Lundmark K, Carlsen J, Rådegran G. Can plasma ADAMTS13 differentiate patients with pulmonary arterial hypertension from other forms of pulmonary hypertension and dyspnea controls. *Chest Pulmonary*. 2025, 3:3, 100178.

Supplement: external discussions of the research in the present thesis

Discussions of the research presented in this dissertation in external, independent publications by other authors. These publications are attached as supplementary material at the end of the thesis, with permission from the publisher.

Editorial discussing paper IV:

- Guazzi M, Bursi F, Rusconi F. Lung Biomarkers: a new route for distinguishing pulmonary hypertension due to HFpEF from pulmonary arterial hypertension. *Int. J. Cardiol.* 2022;351:91-92.

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Letter discussing paper IV:

- Zheng H, Liang J, Chen X, Chen X, Chen J, Wang D, Chen R, Zheng Z. Plasma tumour and metabolism related biomarkers differentiate PAH from HFpEF-PH may improve long-term prognosis. *Int. J. Cardiol.* 2022;347:64-65.

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Summary in Swedish
Sammanfattning på svenska

Vänstersidig hjärtsvikt är ett vanligt tillstånd som drabbar cirka 1 – 3% av världsbefolkningen. Pulmonell hypertension (PH) eller förhöjt tryck i lungkretsloppet, delas in i fem olika grupper beroende på bakomliggande orsak. Den form som är sekundär till vänstersidig hjärtsvikt (grupp 2 PH) utgör en vanlig komplikation vid vänstersidig hjärtsvikt och förekommer i upp till 85% av fallen. Både hjärtsvikt och PH kännetecknas av ospecifika symtom såsom andfåddhet, trötthet och nedsatt fysisk förmåga. Förekomsten av PH förvärrar symtombilden avsevärt och är associerad med ökad sjuklighet och dödlighet. Hjärttransplantation förblir den definitiva behandlingen för utvalda patienter med avancerad vänstersidig hjärtsvikt.

I denna avhandling syftade **delarbete I** och **II** till att undersöka de relativa plasmanivåerna för tyrosinkinas- och tumörrelaterade proteiner i relation till hemodynamik vid avancerad hjärtsvikt, före och ett år efter hjärttransplantation. **Delarbete III** fokuserade på att utvärdera de absoluta nivåerna av vaskulära (bland annat tyrosinkinaser) och inflammatoriska proteiner före hjärttransplantation samt vid flera uppföljningstillfällen därefter. I **delarbete IV** analyserades tumörrelaterade proteiner med målet att identifiera markörer som kan särskilja hjärtsvikt med bevarad ejektionsfraktion och PH (HFpEF-PH) från pulmonell arteriell hypertension (PAH, grupp 1 PH). **Delarbete V** hade som syfte att undersöka det prognostiska värdet av tumörrelaterade proteiner vid vänstersidig hjärtsvikt med samtidig PH. Samtliga studier baserades på Lund Cardio Pulmonary Registry (LCPR). Plasmaproteiner analyserades med proximity extension assays (**delarbeten I, II, IV, V**) eller multiplex sandwich-immunoassays (**delarbete III**). Patienternas hemodynamik undersöktes med högersidig hjärtkateterisering.

I **delarbete I** och **II** påvisades förhöjda plasmanivåer av vascular endothelial growth factor D (VEGF-D), human epidermal growth factor receptor 4, endocan samt brother of CDO hos patienter med avancerad hjärtsvikt med och utan PH. Nivåerna minskade ett år efter hjärttransplantation till värden motsvarande dem hos friska kontrollindivider Minskningen av proteinnivåerna var även förknippad med förbättringar i flertalet hemodynamiska parametrar efter hjärttransplantation. I **delarbete III** minskade de förhöjda absoluta plasmakoncentrationerna av VEGF-D och soluble fms-like tyrosine kinase-1 mest markant under de första fyra veckorna efter hjärttransplantation och fortsatte att sjunka vid sex respektive tolv månaders uppföljning. Proteinerna var även förknippade med flera hemodynamiska parametrar

relaterade till vätskeöverbelastning, PH och hjärtfunktion, både före och vid uppföljningarna efter hjärttransplantation.

I **delarbete IV** kunde en kombination av de tre plasmaproteinerna alfa-1-mikroglobulin/bikunin precursor, lipoprotein lipase och glyoxalase I särskilja HFpEF-PH från PAH med god och högst träffsäkerhet bland de undersökta proteinerna. I **delarbete V** identifierades initialt fem proteiner med koppling till överlevnad och hjärttransplantation hos patienter med vänstersidig hjärtsvikt och PH. Dock försvann denna association efter korrigering för ålder, kön, diabetes typ 2 och förmaksflimmer.

Denna avhandling belyser plasmaproteiner kopplade till signalvägar inom tyrosinkinas, inflammation och tumörbiologi vid avancerad hjärtsvikt, PH, och efter hjärttransplantation. Ytterligare forskning kring dessa proteiner kan fördjupa vår patofysiologiska förståelse om sjukdomarna, underlätta diagnostiken av HFpEF-PH samt hemodynamisk monitorering vid avancerad hjärtsvikt, och efter hjärttransplantation.

Enklare differentiering av HFpEF-PH från PAH kan möjligen bidra till att minska fördröjningen till diagnos av respektive sjukdom och vägleda till korrekt användningen av PAH-specifika behandlingar. Förbättrade möjligheter till hemodynamisk övervakning med biomarkörer skulle dessutom kunna möjliggöra tidigare upptäckt av kliniska försämringar hos patienter med hjärtsvikt, men även av de transplanterade hjärtan som kan vara svåra att upptäcka. Detta behöver dock studeras i framtida projekt.

مُلخَّص باللغةِ العربية Summary in Arabic

يُعَدّ فشل القلب الأيسر من الحالات الشائعة التي تؤثر على واحد إلى ثلاثة في المئة من السكان على مستوى العالم. ارتفاع ضغط الدم الرئوي أو فرط ضغط الدم الرئوي يُصنف إلى خمس مجموعات بناءاً على السبب الكامن وراء المرض. فرط ضغط الدم الرئوي الناتج عن فشل القلب الأيسر (المجموعة الثانية) يعد كمضاعفة لهذا الفشل، ويصيب ما يصل إلى خمسة وثمانين بالمئة من حالات فشل القلب الأيسر. يتصف كل من فشل القلب وفرط ضغط الدم الرئوي بأعراض غير نوعية كضيق التنفس والتعب وانخفاض القدرة البدنية، ويزيد وجود ارتفاع ضغط الدم الرئوي من حدة هذه الأعراض ويؤدي إلى زيادة في معدل الوفيات. لا تزال عملية زراعة القلب هي العلاج الحاسم للمرضى الذين يعانون من فشل القلب الأيسر المتقدم.

وفي هذه الأطروحة المكونة من خمس دراسات، هدفت الدراستان الأولى والثانية إلى تقييم تغير مستويات البروتينات المرتبطة بإنزيم التيروسين كيناز والبروتينات المرتبطة بالأورام في البلازما، وعلاقتها بالديناميكة الدموية في حالات فشل القلب الأيسر المتقدم، وبعد مرور عام من زراعة القلب. أما الدراسة الثالثة فركزت على تقييم المستويات المطلقة للبروتينات الوعائية والالتهابية في الدم قبل زراعة القلب وفي فترات متتابعة بعد ذلك. وشملت الدراسة الرابعة تحليل البروتينات المرتبطة بالأورام في الدم للتميز ما بين فشل القلب الأيسر مع الكسر القذفي المحفوظ مع ارتفاع ضغط الدم الرئوي (المجموعة الثانية)، وفرط ضغط الدم الشرياني الرئوي الأولي (المجموعة الأولى). أما الدراسة الخامسة فكانت تهدف إلى تقييم القيمة التنبؤية للبروتينات المرتبطة بالأورام في فشل القلب الأيسر المصحوب بارتفاع ضغط الدم الرئوي. واعتمدت جميع الدراسات على سجل لوند للقلب والرئة، حيث تم تحليل البروتينات في البلازما باستخدام تقنيات خاصة. كما تم تقييم مستويات الضغط في أنحاء مختلفة في القلب و الأوعية الرئوية (الديناميكة الدموية) للمرضى باستخدام القسطرة القلبية اليمنى.

وأظهرت الدراستان الأولى والثانية ارتفاعًا في مستويات البروتينات VEGF-D و HER4 و Endocan و BOC لدى مرضى فشل القلب. وبعد عام من زراعة القلب انخفضت هذه المستويات لتصل إلى معدلات مشابهة للأشخاص الأصحاء، وكان الانخفاض مرتبطًا بتحسن الديناميكة الدموية. وفي الدراسة الثالثة، حصل انخفاض ملحوظ في مستويات البروتينين VEGF-D و sFlt-1 في البلازما في الأسبوع الرابع بعد زراعة القلب، واستمرت المستويات بالانخفاض عند متابعة المرضى بعد ستة اشهر واثني عشر شهرًا. وبالإضافة إلى ذلك، ارتبط الانخفاض في نسبة البروتينين قبل وخلال السنة الاولى بعد زراعة القلب بعدة مؤشرات ديناميكية دموية تتعلق بالاستسقاء وارتفاع ضغط الدم الرئوي ووظائف القلب .وفي الدراسة الرابعة، استطاعت مجموعة من ثلاثة بروتينات في البلازما AMBP وLPL وGlyoxalase I ان تفرق بدقة عالية بين فشل القلب الأيسر مع الكسر القذفي المحفوظ مع ارتفاع ضغط الدم الرئوي وفرط ضغط الدم الشرياني الرئوي الأولي. وأما في الدراسة الخامسة، فقد تم التعرف على خمسة بروتينات ترتبط بمسار المرض لدى مرضى فشل القلب الأيسر مع ارتفاع ضغط الدم الرئوي. ولكن بعد إجراء المزيد من الدراسات والتمعن في النتائج، اختفت هذه العلاقة.

وملخص ما ذكر، فإن هذه الأطروحة سلطت الضوء على البروتينات المرتبطة بإنزيم التيروسين كيناز و الأورام في فشل القلب المتقدم وفرط ضغط الدم الرئوي، وما بعد زراعة القلب. ويمكن للدراسات الواردة في هذه الأطروحة أن تدعم الأبحاث الأخرى لفهم آليات هذه الأمراض بدقة أكبر و أن تيسر تشخيص فشل القلب الأيسر مع الكسر القذفي المحفوظ مع ارتفاع ضغط الدم الرئوي (المجموعة الثانية) من بين مرضى فرط ضغط الدم الشرياني الرئوي الأولي (المجموعة الأولى). وبالإضافة إلى ذلك، يمكن لهذه الدراسات تسهيل متابعة الديناميكة الدموية لدى مرضى فشل القلب المتقدم وبعد الزراعة بتحليل دم بسيط، مما قد يساعد في اكتشاف التدهور المبكر لدى المرضى و توفير العلاج السريع، سواءً في حالات فشل القلب الأيسر أو بعد زراعة القلب.

Abbreviations

ACC	American College of Cardiology
AHA	American Heart Association
AMBP	Protein AMBP or alpha-1-microglobulin/bikunin precursor
AU	Arbitrary units
BOC	Brother of CDO
BSA	Body surface area
CO	Cardiac output
EDTA	Vacutainer ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
ESC/ERS	European Society of Cardiology/European Respiratory Society
FDR	False discovery rate
FGF-23	Fibroblast growth factor 23
HER4	Human epidermal growth factor receptor 4
HF	Heart failure
HFSA	Heart Failure Society of America
HT	Heart transplantation
HFpEF-PH	Heart failure with preserved ejection fraction and pulmonary hypertension
HFrEF-PH	Heart failure with reduced ejection fraction and pulmonary hypertension
IGF1R	Insulin-like growth factor 1 receptor
IGFBP7	Insulin-like growth factor-binding protein 7
IQR	Interquartile range
LCPR	Lund Cardio Pulmonary Registry
LHF-PH	Left heart failure with pulmonary hypertension (group II)
LPL	Lipoprotein lipase
LVEF	Left ventricular ejection fraction
LVSWI	Left ventricular stroke work index
mPAP	Mean pulmonary arterial pressure
MRAP	Mean right atrial pressure

NPX	Normalised protein expression
NT-proBNP	N-terminal pro-B-type natriuretic peptide
PAC	Pulmonary arterial compliance
PAH	Pulmonary arterial hypertension
PAWP	Pulmonary arterial wedge pressure
PH	Pulmonary hypertension
PH-LHD	Pulmonary hypertension associated with left heart disease
PVR	Pulmonary vascular resistance
RHC	Right heart catheterisation
sFlt1	Soluble fms-like tyrosine kinase-1
sRAGE	Soluble receptor for advanced glycation end products
SV	Stroke volume
SVI	Stroke volume index
sVEGFR-1	Soluble vascular endothelial growth factor receptor 1
VEGF-D	Vascular endothelial growth factor D
WHO	World health organisation
WU	Wood units

Introduction

A historical preface: from dropsy to probing of the right heart

Derived from ancient Greek, "dropsy", the word for water, denoted for many centuries a broad spectrum of conditions characterised by fluid retention or generalised swelling.[1] Dropsical swellings were discussed as early as 2600 B.C in China, as found in *the Yellow Emperor's Classic of Internal Medicine*.[2] Bloodletting was a common treatment for dropsical swellings, and was practiced by the Egyptians and Greeks, and further endorsed in medieval times and thereafter.[1] Moreover, apart from bloodletting, treatment with sweating or purgatives were used by the Egyptians, as described in *the George Ebers Papyrus* in 1500 B.C (**Figure 1**).[1] Likewise, adapted from the Greeks, the Romans reported similar practices, but also advocated paracentesis and suggested that the *"… head in bed should be kept raised"*.[1] In medieval times, the Arab physicians Al-Zahrawi and Ibn-Sina employed cauterisation as a "rescue" therapy in extreme cases when other treatments proved ineffective (**Figure 2**).[1, 3] The practice of bloodletting as the primary treatment continued well into the middle of the 20th century.[1]

While theoretical understanding of cardiovascular physiology had progressed significantly, the assortment of invasive diagnostic methods remained limited.[1, 3, 4] Published in 1929 in the prestigious journal *"Klinische Wochenschrift"*, the article entitled *"Die Sondierung des rechten Herzens" ("Probing of the right heart")* recounts one of the most extraordinary experiments in medical history, in which the young German Werner Forssmann, for the first time known to man, catheterised his own right heart using a ureteric catheter.[5, 6] Though a urological surgeon and not a cardiologist, he was fascinated by the innovative work of the French physiologists Étienne-Jules Marey and Claude Bernard, who performed heart catheterisation on horses in the latter half of the 19th century.[5] Forssmann's publication, in subsequent years, prompted the early catheterisation studies by André Cournand and Dickinson Richards, both of whom were pulmonologists interested in acquiring mixed venous blood samples.[5] Their rationale was based on the belief that *"Because it is apparently the soundest method for obtaining mixed venous blood for respiratory gas determinations, and because of the numerous problems of hemodynamics it might help solve…"* not fully anticipating the implications their work would have on revolutionising the field of cardiology.[5, 7] In following years, they advanced the catheters sequentially into the right atrium, right ventricle and finally the pulmonary artery.[5] In 1945, Cournand and his colleagues succeeded to record the pulmonary

arterial pressures utilising a specially designed double-lumen catheter with two distal orifices, one in the pulmonary artery and the other in the right ventricle attached to a Hamilton manometer. This was demonstrated on a patient with severe pulmonary hypertension and rheumatic valvulitis, most likely a severe mitral stenosis (**Figure 3**). Later, Werner Forssmann, together with André Cournand and Dickinson Richards were awarded the Nobel Prize for Medicine or Physiology in 1956.[5]

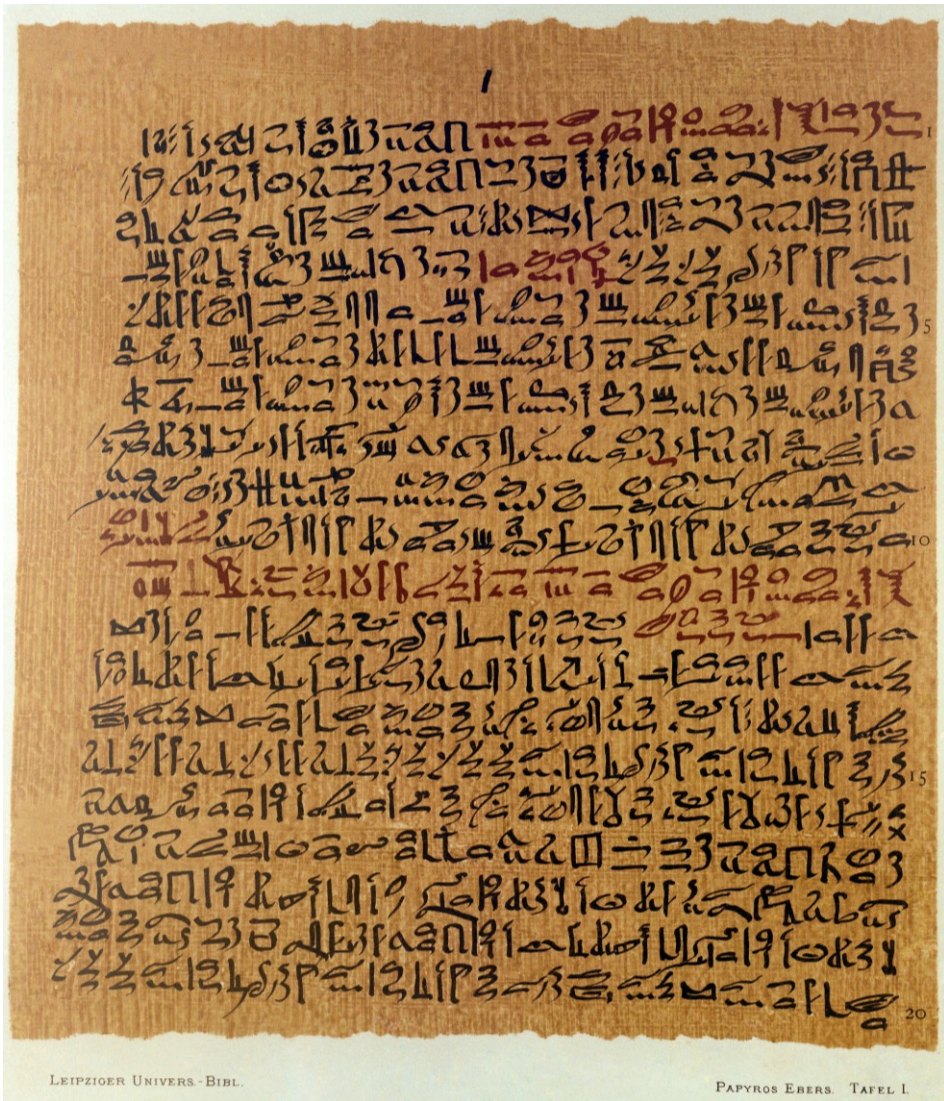

Figure 1.
George Ebers Papyrus, one of the oldest and most comprehensive medical papyri from ancient Egypt, dating back to approximately 1500 B.C. This page is written in hieratic script, a cursive form of Egyptian hieroglyphs.[8]

Figure 2.
"Kitab al-Tasrif li-man 'ajiza 'an al-ta'lif" ("Treatise on Surgery"), the original Arabic work by Abu'l-Qasim al-Zahrawi (Latin: Albucasis) illustrating and describing surgical instruments he devised in the 13th century.[9]

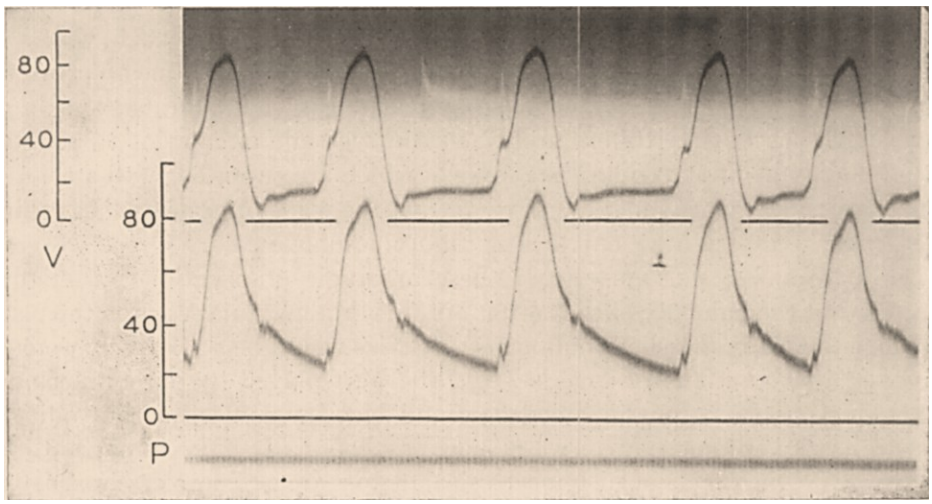

Figure 3.
Photographic reproduction of the first pulmonary arterial pressures recorded in man (upper curve), with simultaneous measurement of the right ventricular pressures (lower curve). These pressure tracings, measured in mmHg, were obtained using a double lumen catheter in a patient with rheumatic valvulitis and cardiac decompensation, displaying severe pulmonary hypertension, originally published in 1945. Reproduced with permission (license: 6040721073002).[10]

Heart failure

The earliest identified case of "acute decompensated" heart failure (HF) using forensic analysis is probably the mummified remains of Nebiri, an Egyptian dignitary who lived during the reign of Pharaoh Thutmose III (1479 – 1424 B.C).[2, 11] Long after, in the 17th century, significant advances were made in the understanding the cardiopulmonary physiology.[4, 12, 13] This progress was later followed by the introduction of invasive haemodynamic assessments, which made it possible to understand that congestion, irrespective of HF aetiology, was due to increased intracardiac filling pressures.[14]

Definition, classification and staging

Definition

HF is a complex syndrome characterised by diminished exercise capacity, poor quality of life, and high mortality.[15] Over the past decades, various definitions have been postulated to define this heterogeneous condition. Some of these definitions integrate medical history with clinical presentation, physical examination and laboratory findings, whereas others emphasise the haemodynamic and physiological aspects; each characterised by distinct advantages and limitations.[16, 17] In 2021, the American, European, and Japanese HF societies proposed a unified definition of HF as *"a clinical syndrome with symptoms and or signs caused by a structural and/or functional cardiac abnormality and corroborated by elevated natriuretic peptide levels and or objective evidence of pulmonary or systemic congestion"*.[17]

Classification and staging

During the latter half of the 20th century, determining the fraction of left ventricular (LV) end-diastolic volume ejected during each cardiac cycle (LVEF) was of huge interest to acquire a more complete understanding of the LV physiology.[16, 18] In the early invasive studies, before the emergence of echocardiography, patients with decompensated cardiac function and poorer prognosis presented with lower LVEF compared to healthy individuals.[16] This was further demonstrated in the post-hoc analyses of the *Veterans Administration Cooperative Study* (V-HeFT), in which patients with lower LVEF had poorer prognosis, resulting in changes in the designs of subsequent mortality trials in HF.[18-21]

Compared to invasive measurements, echocardiographic assessment of LVEF is extremely load-dependent, and may be influenced by geometric alterations, such as in physiological athletic-, hypertrophic- and dyssynchronous LV states. LVEF may also be underestimated during tachycardia, and do neither correlate with New York Heart Association (NYHA) classes nor correspond to true LV contractility.[19, 22] Accordingly, LVEF may more accurately be viewed as a measure of ventricular-arterial coupling rather than an indicator of systolic function.[23] Despite these shortcomings which

substantially affect LVEF reproducibility, classification based on LVEF has traditionally been, and is still adapted due to the evidence of benefit in outcomes in clinical trials of HF with reduced LVEF, in addition to its practical and convenient non-invasive way of measuring.[16, 19]

In the unified statement, HF is classified based on LVEF into: HF with reduced ejection fraction (HFrEF, LVEF ≤ 40%), mildly reduced ejection fraction (HFmrEF, LVEF 41 – 49%), and preserved ejection fraction (HFpEF, LVEF ≥ 50%).[17] Additionally, patients with an initially reduced LVEF (≤ 40%) who subsequently demonstrate an absolute increase in LVEF of at least 10% compared to baseline are defined as HF with improved ejection fraction (HFimpEF).[17] Apart from minor differences, these classifications align with both the 2022 American Heart Association, American College of Cardiology, and Heart Failure Society of America (AHA/ACC/HFSA) guidelines and the 2021 and the revised 2023 European Society of Cardiology (ESC) HF guidelines.[24-26] Rather than representing a distinct fourth category, HFimpEF is considered a subclass of HFrEF in the American HF guidelines, without specifying the degree of improvement required for LVEF.[17, 24] The ESC HF guidelines acknowledge HFimpEF as a separate entity from HFpEF.[25, 26]

The NYHA classification, often integrated in other stagings, is widely used and describes symptoms and functional capacity in HF.[24, 27] Staging of HF (A to D) constitutes an important clinical asset, reflecting the evolution and progression of the disease, in which more advanced stages (C and D) are associated with worse survival.[17, 24, 28, 29] Stage-directed therapeutic interventions are recommended to prevent HF onset, alleviate symptoms, and to reduce morbidity and mortality.[24, 29] Advanced HF, or stage D, comprises 1 to 10% of all cases of HF and defines an important clinical group of patients who may benefit from more advanced therapies including mechanical circulatory support and heart transplantation (**Figure 4**).[24, 25, 30] According to the latest statement of the ESC Heart Failure Association in 2018, advanced HF is characterised by severe and persistent symptoms (NYHA III or IV), severe cardiac dysfunction, recurrent episodes of systemic pulmonary congestion requiring inotropic support or intravenous diuretics as well as severe impairment in exercise capacity due to cardiac failure.[24, 25, 30] Further classification using The Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) profiles provides prognostic information and guide clinical decision-making regarding pacing therapies, mechanical support, and heart transplantation in advanced HF.[25, 31-33]

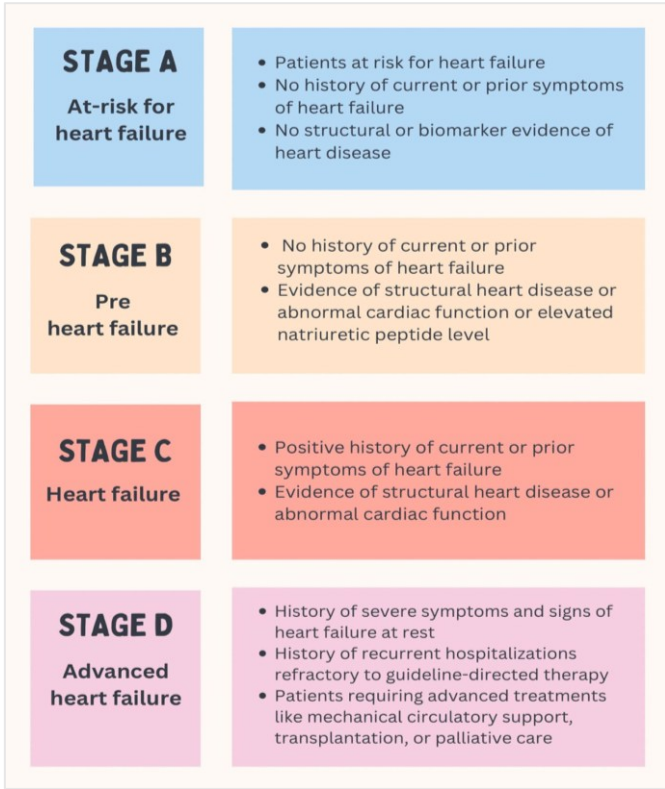

Figure 4.
Staging (A to D) of heart failure (HF), in which A and B are preclinical, whereas C and D represent overt HF with increasing severity from C to D. The stages reflect the development and progression of HF according to the 2022 American Heart Association, American College of Cardiology, and Heart Failure Society of America (AHA/ACC/HFSA) HF guidelines. Abbreviations: CVD indicates cardiovascular disease; and GDMT, guideline-directed medical therapy. Reproduced with permission (6064720474326).[24, 34]

Epidemiology and aetiology

HF constitutes a major health and socioeconomical burden, owing to its high prevalence, hospitalisation rates, health care expenditures and mortality, affecting at least 64.3 million people worldwide.[15, 25, 35] The overall estimated prevalence of HF in adults ranges from 1 to 3% in developed countries globally, increasing to > 10% in those aged ≥ 70 years, and is steadily increasing.[15, 25] When stratified by LVEF, the prevalence estimates range from 32 to 62% for HFrEF, 14 to 24% for HFmrEF, and 16 to 52% for HFpEF.[15] Over time, while the prevalence appear to be stable or even declining for HFrEF, it is increasing for HFpEF.[15, 25] Conversely, the incidence of HF, estimated at 3 to 6 cases per 1000 person years, is declining in developed countries. This is most likely due to improved preventive care, as well as better detection and management of comorbid risk factors (**Figure 5**).[15, 25, 36, 37]

The leading aetiologies of HF vary across regions with racial and ethnic disparities, and most data is available from developed and western-like countries.[15, 24, 25] The most common causes include ischaemic heart disease and coronary artery disease, systemic hypertension, and valvular- and rheumatic heart disease, accounting approximately for 40%, 15%, and 11% of the HF cases worldwide, respectively.[15, 38] In addition to congenital-, metabolic- and endocrine- disorders, other causes include idiopathic dilated cardiomyopathy, as well as toxin-, drug-, genetic-, radiotherapy-, and stress-induced cardiomyopathies. [15, 24] In the Sub-Saharan region and low-income countries, rheumatic heart disease represent a major aetiology, whereas Chagas cardiomyopathy, caused by the parasite *Trypanosoma cruzi*, remains the most common cause of non-ischaemic cardiomyopathy in South America.[15]

The prognosis of HF remains poor, with high mortality rates and compromised quality of life. In observational studies, the estimated one-year mortality of chronic HF ranges from 7% to 15%.[15, 39] Most estimates are, however, limited by older data from populations not treated with modern therapies and may thus not accurately reflect contemporary mortality trends in HF. Compared to HFrEF, HFmrEF and HFpEF appear to have higher survival rates, however, further studies are warranted to more precisely define these outcomes.[15, 25]

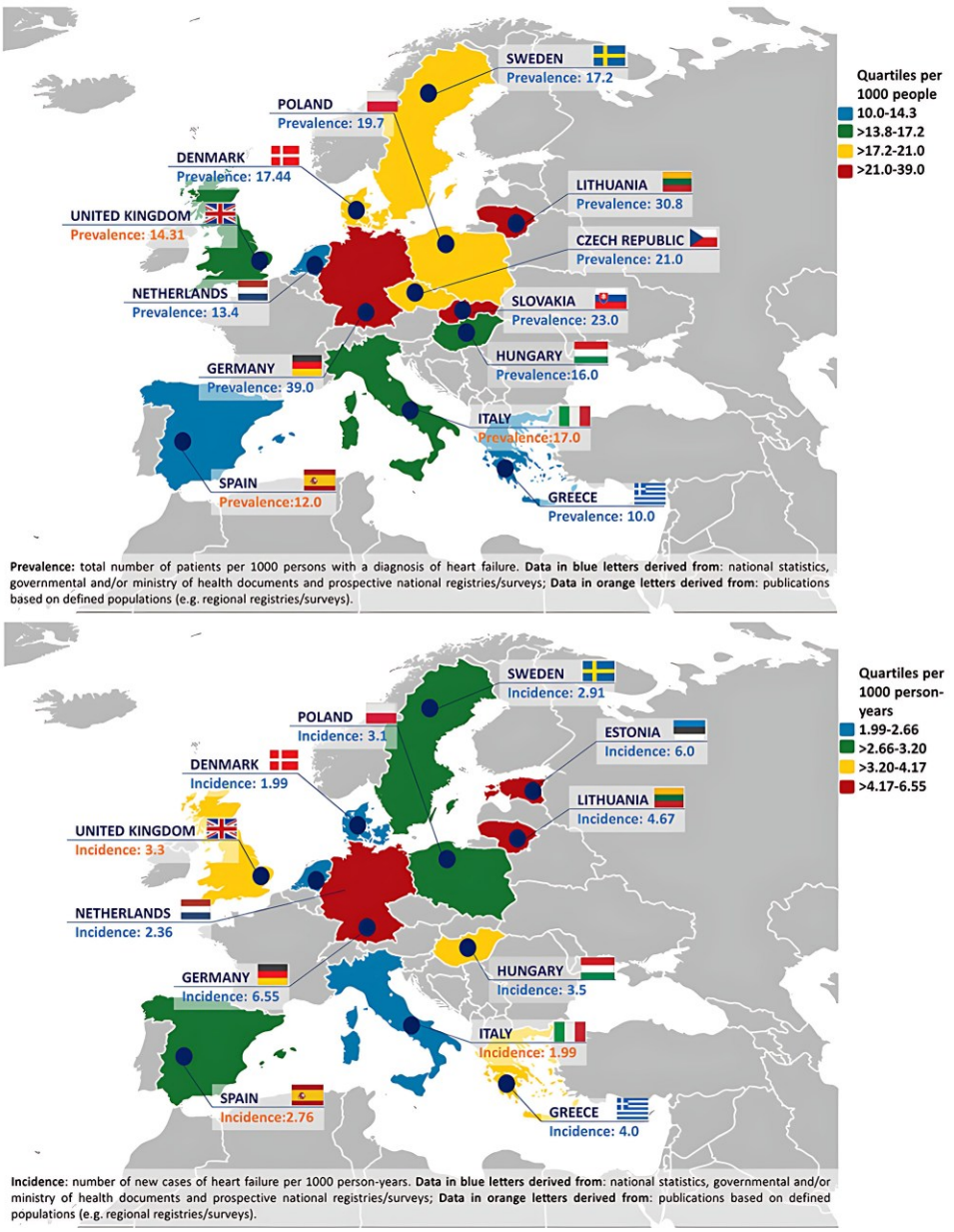

Figure 5.

Geographical representation of the prevalence and incidence of HF across different countries in Europe, according to The Heart Failure Association Atlas in 2019. Reproduced with permission (license: 0006045250149087).[37]

Pathophysiology

The clinical syndrome of HF arises due to structural and functional alterations leading to impaired cardiac function, which is characterised by an inability to maintain adequate organ perfusion commensurate with the requirements of the metabolising tissue, or to do so only with increased filling pressures.[14] HF is a progressive systemic disorder with heterogenous pathophysiology.[25, 40, 41] The development of HF, regardless of aetiology, is preceded by myocardial injury, after which compensatory mechanisms are triggered from a macrostructural to molecular level. These mechanisms act to maintain cardiac haemostasis and adequate physiological functioning.[40] Although initially beneficial, over time, these processes lead to impaired contractility, excessive volume and pressure overload and reduced organ perfusion. These compensatory mechanisms are influenced by non-modifiable factors such as age, sex and genetics.[40, 42]

Left ventricular function, remodelling and vascular changes

The LV function is determined by preload, afterload and myocardial contractility, which are influenced by Frank-Starling mechanism (increased preload to a certain point will increase the stroke volume) along with Laplace's law (increased myocardial oxygen demand due to elevated ventricular wall stress) (**Figure 6**).[40, 42, 43]

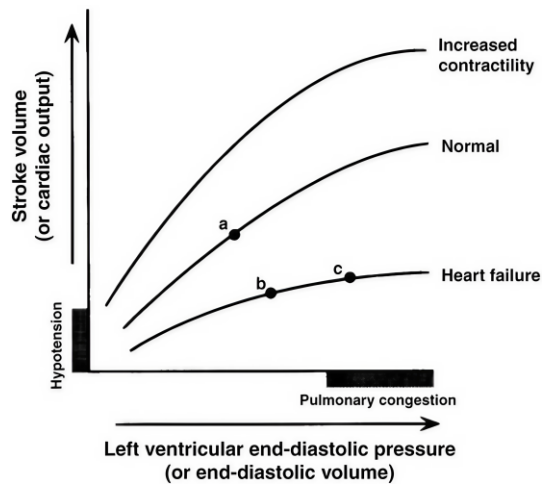

Figure 6.

Three Frank-Starling curves depicting stroke volume as a function of left ventricular end-diastolic pressure or preload, in physiological states and in heart failure. During ventricular filling in end-diastole, the maximal myocardial stretch lengths are determined by myocardial compliance (distensibility), the filling volume (preload) and the resting force. The middle curve represents a healthy heart (a), in which an increase in preload is followed by a corresponding increase in stroke volume (due to stretch in the myocardium), which reaches a plateau at a certain preload. The lower curve, representing a failing heart with systolic dysfunction, is flattened and lies below that of the healthy heart due to diminished cardiac reserve. In response to increased preload at point (b), there is still a compensatory increase in stroke volume. Further increase in preload beyond point (c) will lead to pulmonary congestion. Over time, the curve will flatten further and slope downwards as heart failure progresses and compensatory mechanisms become exhausted. Reproduced with permission (license: 6046401094250).[42]

Cardiac remodelling can be classified into physiologic (adaptive) or pathologic (maladaptive), involving all structural components of the heart.[40] In maladaptive cardiac remodelling, the macrostructural alterations are characterised by LV hypertrophy and dilatation due to reorganisation and elongation of the cardiomyocytes, increased ventricular wall tension with impaired perfusion of the subendocardium, as well as increased ventricular stiffness due to elevated filling pressures resulting in diastolic dysfunction (**Figure 7**).[40, 44] As the LV progressively dilates, atrial- and papillary muscle- misalignment may develop, leading to mitral valve regurgitation. These alternations are further accompanied by contractile dyssynchrony, resulting in impaired contractile efficiency.[40, 42]

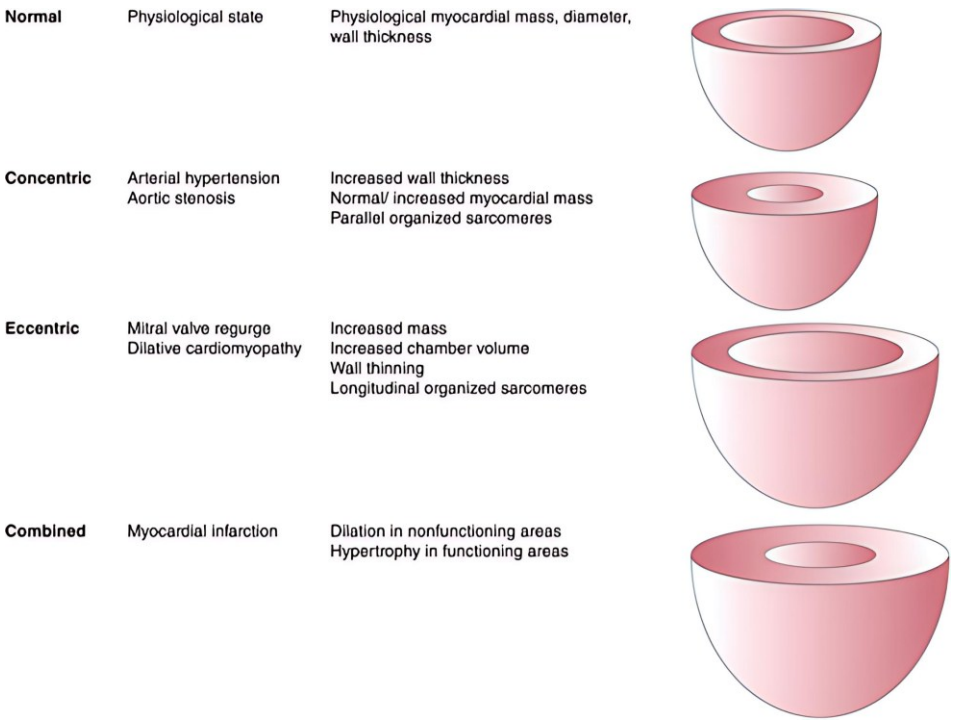

Figure 7.

Geometric representation of left ventricular hypertrophy and remodelling in response to different haemodynamic states. In heart failure, concentric hypertrophy develops as a result of pressure overload, commonly observed in diastolic dysfunction, whereas sustained volume overload leads to eccentric hypertrophy.[40] Left ventricular hypertrophy manifests as **i.)** concentric hypertrophy (due to pressure overload, with both increased ventricular mass and relative wall thickness), **ii.)** eccentric hypertrophy (due to volume overload, with increased ventricular mass, but with either increased, normal or decreased relative wall thickness depending on underlying aetiology), or **iii.)** combined concentric-eccentric hypertrophy (which may occur following myocardial infarction). Over time, the overall left ventricular geometry progressively shifts from an elliptical to a more spherical shape, which is associated with detrimental consequences including impaired contractility.[42] Reproduced with permission (license: 6046450196015).[40]

Apart from cardiomyocyte hypertrophy, the microstructural changes involve an accelerated cycle of regeneration, apoptosis and necrosis, resulting in slower cellular turnover and impaired myocyte progenitor cell function. This is accompanied by a shift towards soluble collagen deposition in the interstitium, resulting in reduced myocardial cross-linking, thereby impairing LV contractility and compliance. This contributes to further hypoxia-induced structural changes due to reduced capillary density.[40, 44] These changes are accompanied by vascular endothelial dysfunction, increased systemic vascular resistance, higher venous pressure, and arteriolar constriction, ultimately impairing organ perfusion.[40, 42]

The neurohormonal and other signalling pathways

The progression, and extent of cardiac and vascular remodelling as well as their functional consequences, are further affected by the severity, type, and persistence of myocardial injury. These changes are influenced by multiple mechanisms, resulting in phenotypic cardiac alterations.[40-42] Key mechanisms include activated neurohormonal, inflammatory and immunomodulatory pathways, increased oxidative stress, as well as deranged cardiac metabolism and energetics (**Figure 8**).[40, 42, 45]

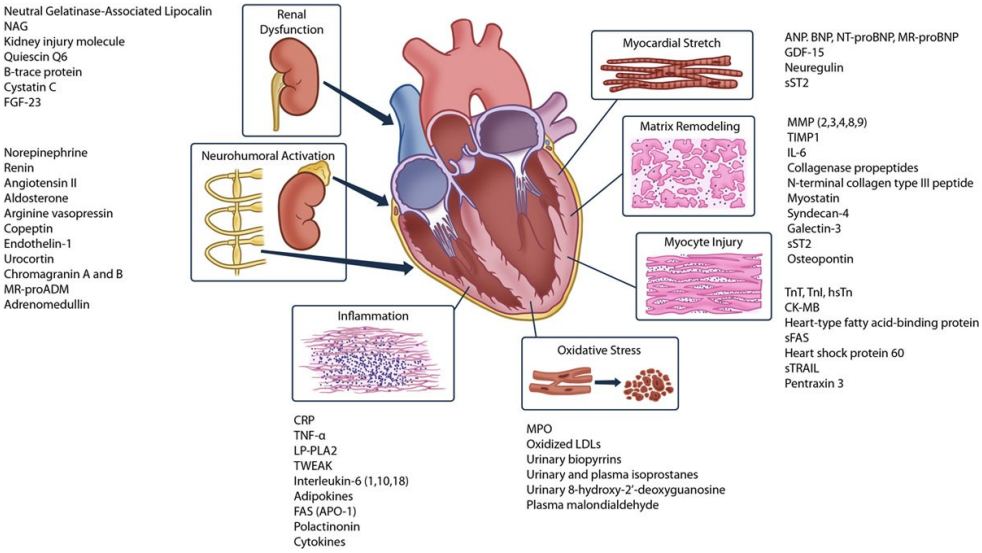

Figure 8.

Mechanistic insights into the pathology of heart failure and associated biomarkers representing different pathways. **Renal dysfunction**: FGF-23 indicates fibroblast growth factor 23; and NAG, N-acetyl-β-D-glucosaminidase; **Neurohormonal activation**: MR-proADM, midregional proadrenomedullin; **Inflammation**: CRP, C-reactive protein; FAS, Fas cell surface death receptor; LP-PLA2, lipoprotein-associated phospholipase A2; and TWEAK, tumor necrosis factor-like weak inducer of apoptosis; **Oxidative stress**: LDL, low-density lipoprotein; and MPO, myeloperoxidase; **Myocyte injury**: CK-MB, creatine kinase-muscle/brain; sFAS, soluble Fas cell surface death receptor; sTRAIL, soluble TNF-related apoptosis-inducing ligand; hsTn, high-sensitivity troponin; TnI, troponin I; and TnT, troponin T; **Matrix remodelling**: IL-6, interleukin 6; MMP, matrix metalloproteinase; sST2, soluble suppression of tumorigenicity 2; and TIMP1, tissue inhibitor of metalloproteinases 1; **Myocardial stretch**: ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; GDF, growth differentiation factor; MR-proBNP, midregional pro-B-type natriuretic peptide; and NT-proBNP, N-terminal pro-B-type natriuretic peptide. Reproduced with permission (license: 6046510653622).[45]

The neurohormonal axis involves the sympathetic nervous system, the renin-angiotensin-aldosterone system, and the vasopressin system.[40, 42, 46] The neurohormonal system acts to maintain adequate perfusion pressure by increasing total peripheral resistance through the release of catecholamines. This along with sodium and water retention to increase preload and maximise stroke volume via the Frank-Starling mechanism.[40, 46] The reduction in flow and mean arterial pressure (MAP) in HF (sensed by high-flow baroreceptors in the carotid sinuses and the aortic arch) stimulates the release of catecholamines, which in turn lead to central vasodilation, peripheral vasoconstriction, and positive inotropic- and chronotropic effects.[40, 42, 46, 47] Conversely, catecholamine-mediated cardiac stimulation is proarrhythmic, and leads to adverse cardiac remodelling, promoting cardiomyocyte hypertrophy and apoptosis.[40, 42]

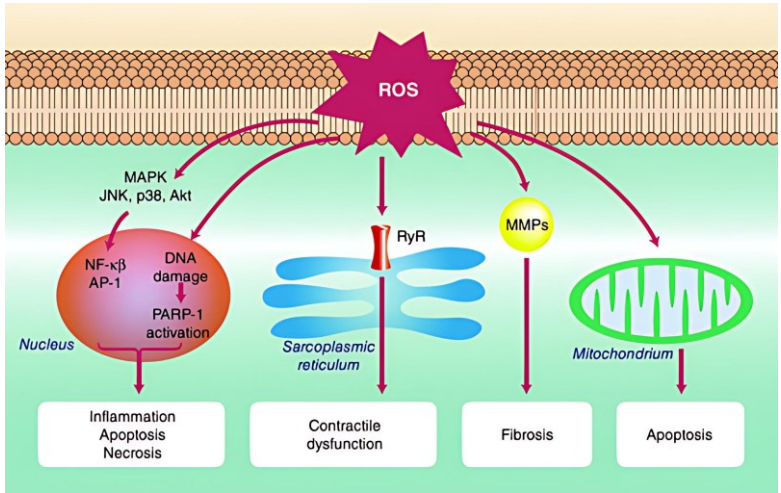

Figure 9.

The role of reactive oxygen species (ROS) and their adverse intracellular signalling and consequences in cardiac cells during heart failure. Abbreviations Akt indicates protein kinase B; AP-1, activator protein 1; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MMPs, matrix metalloproteinase; NF-κβ, nuclear Factor kappa-light-chain-enhancer of activated B cells; PARP-1, Poly ADP-ribose polymerase 1; and RyR, Ryanodine Receptor. Reproduced with permission (license: 6046400327381).[40]

Moreover, the baroreceptor-mediated responses trigger arginine vasopressin secretion, which in turn promotes water reabsorption and induces systemic vasoconstriction.[40] The subsequent superimposed pressure- and volume overload stimulate myocardial stretch and the release of natriuretic peptides, which promotes vasodilation, natriuresis and suppression of adrenergic responses.[40, 42, 46] The consequent reduction in renal perfusion promotes activation of the renin-angiotensin-aldosterone system, leading to increased sodium and water reabsorption into the systemic circulation, vasoconstriction, and increased LV afterload, the latter increasing energy demands.[40, 42] Deranged energetics manifest as ineffective substrate utilisation, characterised by a shift towards glucose metabolism and impaired energy production. The resultant mismatch between oxygen supply and demand leads to the accumulation of reactive oxygen species, damaging the mitochondria and the transfer of energy substrates.[40] The increase in oxidative stress in the myocardial milieu is also believed to arise from autooxidation of catecholamines, repetitive ischaemic insults and reperfusions, as well as activation of proinflammatory- and redox-sensitive pathways (**Figure 9**).[40]

Heart failure with preserved ejection fraction

LV diastolic dysfunction is characterised by increased passive viscoelastic chamber stiffness, slower active relaxation, or both, impairing filling.[23] The consequent elevation of LV filling pressures at rest and/or during exercise promotes the unspecific symptoms of HF.[23, 48] A decline in LV compliance and relaxation may, however,

manifest as a part of the normal ageing, or secondary to cardiac and metabolic comorbidities such as systemic arterial hypertension, atrial fibrillation, obesity, and diabetes mellitus. However, the presence of diastolic dysfunction does not necessarily indicate a clinically overt HFpEF, nor does it guarantee its future development.[48] Beyond diastolic dysfunction, which is only present in two thirds of patients with HFpEF at rest with echocardiography, non-diastolic abnormalities such as subtle impairment in LV systolic function, reduced systolic reserve capacity, and chronotropic incompetence limit cardiac output reserve and contribute to the syndrome (**Figure 10**). These changes become substantially more pronounced during stress or exercise.[23, 48]

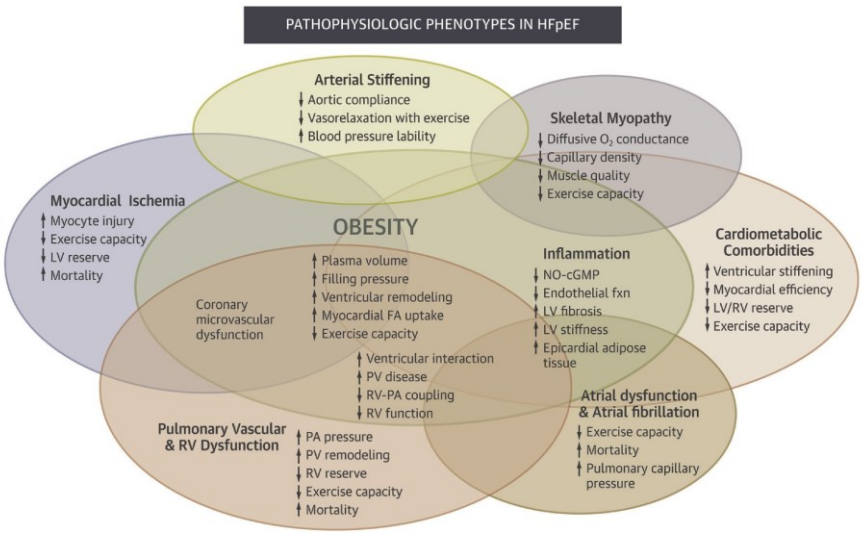

Figure 10.
Insights into the various phenotypes of heart failure with preserved ejection fraction (HFpEF), based on the predominant pathophysiology. HFpEF manifests with various phenotypes, the underlying pathology of which remains to be elucidated. In addition to impaired left ventricular (LV) diastolic function and systolic reserve, ventricular dyssynchrony, relative pericardial constraint, and loss of atrial contractile reserve contribute to the development of HFpEF. Abbreviations: FA indicates fatty acid; fxn, function; NO-cGMP, nitric oxide-cyclic guanosine monophosphate signalling; PA, pulmonary arterial; PV, pulmonary vascular; and RV, right ventricular. Reproduced with permission (license: 6051220905100).[48]

Diagnosis

According to the 2021 ESC HF guidelines, the diagnosis of HF should be based on clinical findings of HF, elevated natriuretic peptides and objective evidence of LV dysfunction (**Figure 11**).[25] This is in line with the 2022 American HF guidelines, which also emphasise the importance of objective evidence of elevated LV filling pressures, either at rest or during exercise/provocative testing.[24]

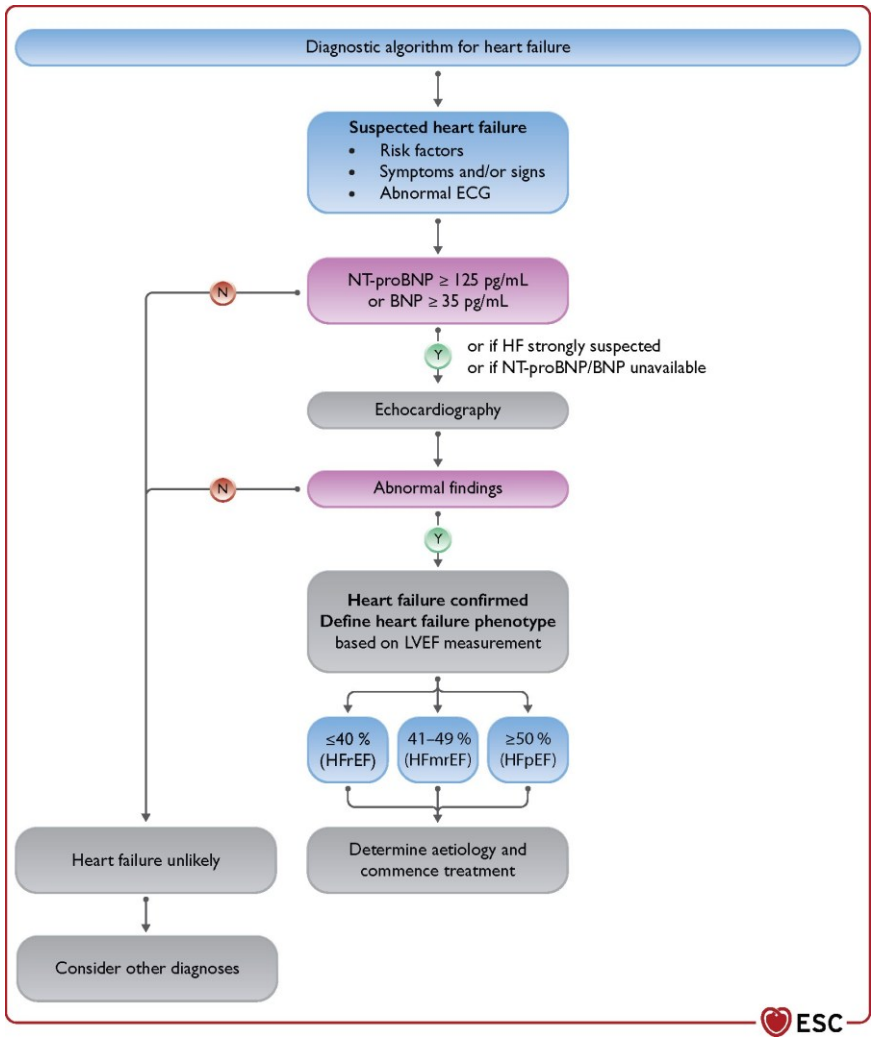

Figure 11.

The 2021 European Society of Cardiology (ESC) diagnostic algorithm of chronic heart failure (HF), and further stratification according to left ventricular ejection fraction (LVEF). Abbreviations: BNP indicates B-type natriuretic peptide; ECG, electrocardiogram; HFrEF, HFmrEF, and HFpEF, heart failure with reduced-, mildly reduced-, and preserved ejection fraction; and NT-proBNP, N-terminal BNP. Reproduced with permission (license: 6057170177266).[25]

As the diagnosis of HFpEF remains challenging, several scores have been proposed to aid the diagnosis. The H_2FPEF score, for instance, was endorsed by the 2022 American HF guidelines to facilitate the evaluation and decision-making (**Figure 12**).[24, 49] The HFA of ESC counterpart, however, recommends the use of HFA-PEFF diagnostic algorithm (**Figure 13**).[50] To facilitate a broader clinical utilisation, the 2021 ESC HF guidelines suggest the initial use of a simplified algorithm, followed by the HFA-PEFF algorithm if additional diagnostic guidance is needed.[25, 50]

	Clinical Variable	Values	Points
H_2	Heavy	Body mass index > 30 kg/m^2	2
	Hypertensive	2 or more antihypertensive medicines	1
F	Atrial Fibrillation	Paroxysmal or persistent	3
P	Pulmonary Hypertension	Doppler echocardiographic estimated right ventricular systolic pressure > 35 mmHg	1
E	Elder	Age > 60 years	1
F	Filling Pressure	Doppler echocardiographic E/e' > 9	1
		H_2FPEF score	Sum (0-9)

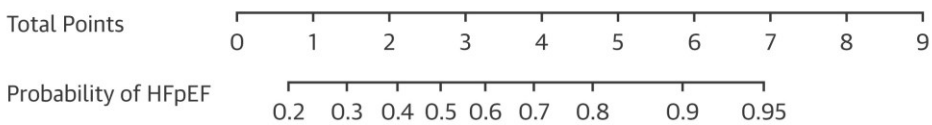

Figure 12.

The nomogram of H_2FPEF, a clinically derived composite score incorporating six readily available variables, in which the odds of having heart failure with preserved ejection fraction (HFpEF) is doubled for each one-unit score increase. The diagnosis of HFpEF from other aetiologies of dyspnoea can be facilitated by H_2FPEF, in which scores < 2, 2-5, and ≥ 6 represent low-, intermediate-, and high likelihood of having HFpEF, respectively. Intermediate likelihood scores (between 2 and 5) warrant further evaluation to confirm or negate the diagnosis of HFpEF. Reproduced with permission (licenses: 6051320828195, and 6051321018699).[48, 49]

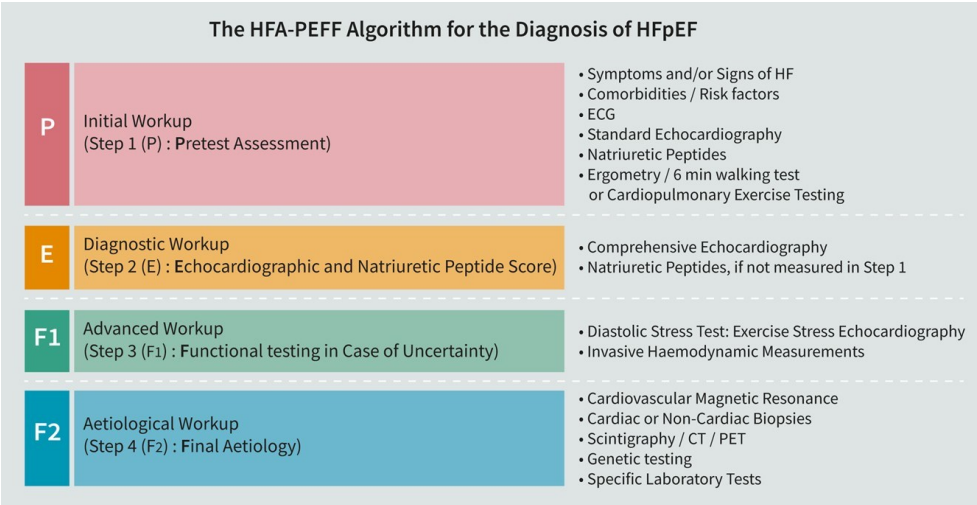

Figure 13.

The Heart Failure Association (HFA) of the European Society of Cardiology (ESC) developed the HFA-PEFF algorithm to facilitate the diagnosis of heart failure with preserved ejection fraction (HFpEF). This stepwise approach comprises four stages: P, E, F1, and F2. Step 1 (P) involves an initial work-up with pre-test assessment of HFpEF. Step 2 (E) involves a comprehensive echocardiographic evaluation. Step 3 (F1) entails advanced testing to assess diastolic dysfunction, which may involve stress echocardiography or right heart catheterisation. Step 4 (F2) incorporates additional studies, which may include cardiac magnetic resonance imaging, endomyocardial biopsies, or genetic testing, to further characterise the underlying aetiology. Abbreviations: CT indicates computed tomography; ECG, electrocardiogram; and PET, positron emission tomography. Reproduced with permission (license: 6055870588821).

Management and treatment

Over the past four decades, significant advances have been made in HF management, and in identifying disease-modifying therapies, with positive impact on outcomes.[24-26]

General measures

Different multidisciplinary models have been postulated in the management of chronic HF, impacting positively on outcome. These programmes facilitate shared decision making, consideration of further treatment escalation, and transition to supportive care.[25] Irrespective of ejection fraction, general aspects include patient education and symptom self-management, optimal treatment of risk factors and comorbidities, regular influenza-, pneumococcal- and COVID-19 vaccinations to reduce hospitalisations, as well as exercise rehabilitation, and the use of diuretics to reduce congestion-related symptoms.[24, 25, 51-54]

Heart failure with reduced ejection fraction (HFrEF)

Treatment in HFrEF aims to enhance functional capacity, improve quality of life, and reduce morbidity and mortality. The use of angiotensin-converting enzyme inhibitors (ACEi), or angiotensin receptor-neprilysin inhibitors (ARNi), beta-blockers, and mineralocorticoid receptor antagonists (MRA) constitute the cornerstone of treatment in HFrEF.[24, 25] These medications (targeting the renin-angiotensin-aldosterone and the sympathetic nervous systems) reduce symptoms, HF-related hospitalisations, and improve survival.[39, 55-65] ARNi may be used as first-line therapy in ACEi-naïve or de novo HF, or as a replacement for ACEi in symptomatic HF with NYHA classes II-III, to further reduce morbidity and mortality.[66, 67] Angiotensin receptor blockers (ARBs) can be used as an alternative in cases of ACEi or ARNi intolerance due to serious adverse effects.[24, 25] The addition of sodium-glucose co-transporter 2 (SGLT2) inhibitors further reduces HF-related hospitalisation rates and cardiovascular mortality.[24, 25, 68, 69]

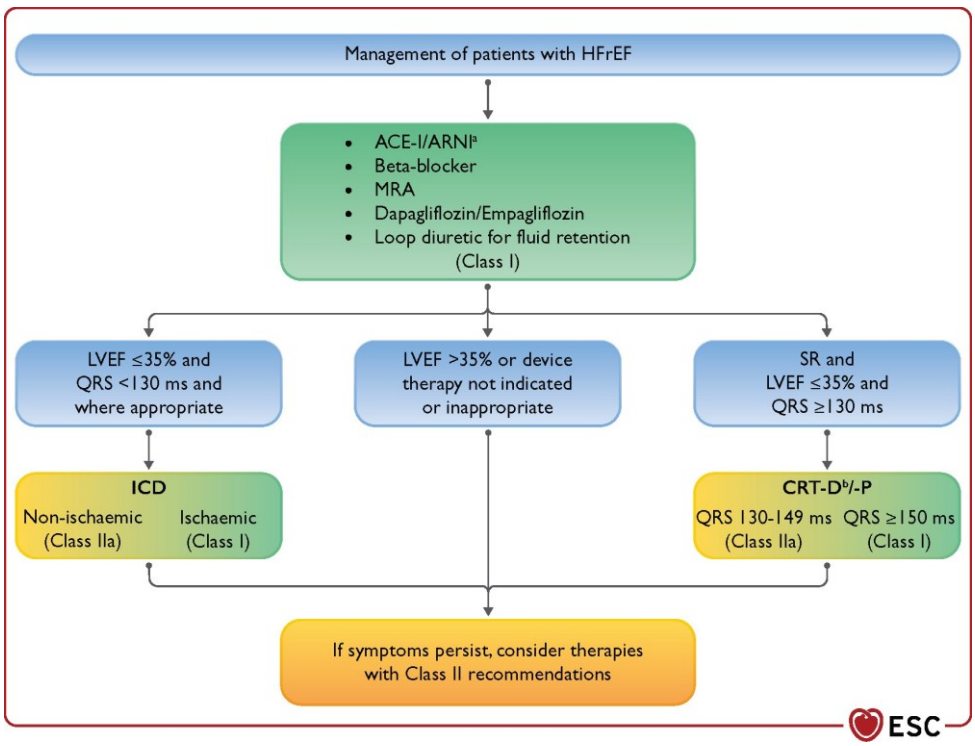

Figure 14.

Treatment algorithm of heart failure with reduced ejection fraction (HFrEF) according to the 2021 European Society of Cardiology HF guidelines. Abbreviations: ACEi indicates angiotensin-converting enzyme inhibitor; ARNi, angiotensin receptor-neprilysin inhibitor; CRT, cardiac resynchronization therapy; ICD, implantable cardioverter defibrillator; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; and SR, sinus rhythm. Reproduced with permission (license: 6057161503170).[25]

Other pharmacological therapies (Ivabradine, Hydral-nitrats, Vericiguat, and Digoxin/Digitoxin) may be considered in selected populations as add-on therapies to ACEi/ARB/ARNi, SGLT2 inhibitors, beta-blockers and MRA after maximum up-titration.[20, 24, 25, 70-83] Ivabradine (an I_f-channel inhibitor) may be considered in patients with symptomatic HF with sinus rhythm (NYHA classes II-III, LVEF ≤ 35%, and a heart rate ≥ 70 beats/min) to further reduce HF hospitalisations and cardiovascular mortality.[24, 25, 70-72] Moreover, in symptomatic African American patients with NYHA classes III-IV, or those intolerant to ACEi, ARB and ARNi, the combination hydralazine and isosorbide dinitrate may be used to reduce HF hospitalisations and mortality.[20, 24, 25, 73, 74] On the other hand, digoxin may be used in patients with sinus rhythm to reduce hospitalisations.[24, 25, 76-82] Among patients receiving guideline-directed medical therapy, treatment with digitoxin, a cardiac glycoside similar to digoxin, was shown in the recent DIGIT-HF trial to reduce the composite endpoint of all-cause mortality or HF hospitalisations compared with placebo.[83] Finally, intravenous iron supplement is recommended in symptomatic patients to alleviate symptoms and increase quality of life.[24, 25, 84, 85]

Beyond optimal pharmacological treatment, cardiac resynchronisation therapy is recommended in symptomatic patients (LVEF ≤ 35%) with sinus rhythm and prolonged QRS duration, or with high-degree atrioventricular block, to reduce morbidity and mortality.[24, 25] Due to the increased risk of sudden death, especially in ischaemic HF aetiology, an implantable cardioverter defibrillator is recommended as a primary or secondary prevention measure when the estimated life expectancy is greater than one year (**Figure 14**).[24, 25, 86-90]

Heart failure with improved ejection fraction (HFimpEF)

To date, this group should be treated as HFrEF. However, there is a paucity of evidence regarding the treatment recommendations for HFmrEF who improve their LVEF to ≥ 50%, and whether they should be treated as HFmrEF or HFpEF.[24, 25]

Heart failure with mildly reduced ejection fraction (HFmrEF)

Since the EMPEROR-Preserved- and DELIVER-trials, SGLT2 inhibitors are now recommended to reduce cardiovascular death or HF hospitalisations.[24, 26, 91, 92] The addition of ACEi/ARB/ARNi, beta-blockers or MRA may be considered, especially in those with LVEF at the lower end.[24, 26] As in HFrEF, intravenous iron supplement is recommended to reduce symptoms and improve quality of life.[24, 26, 93, 94]

Heart failure with preserved ejection fraction (HFpEF)

Treatments differ in this heterogenous group depending on the underlying aetiology. Regardless, SGLT2 inhibitors are indicated to reduce HF-related hospitalisations or cardiovascular deaths.[24, 26, 91, 92] There is, however, no clear indication for the use of ACEi/ARB/ARNi, beta-blockers or MRA to treat the underlying HF.[24, 26] The focus should also be on treating other comorbidities.[24, 26]

Advanced therapies

Mechanical circulatory support systems

Despite optimal medical, surgical (e.g. valve repair) and device therapies, a significant proportion of chronic HF cases progress into advanced disease, with a one-year mortality rate as high as 75%.[24, 95] When these treatment options have been exhausted, implantation of a mechanical circulatory support device may be an alternative.[95-97] Mechanical assist devices are classified by implant location, pump mechanism, implant strategy (bridge to transplant, bridge to candidacy, or destination therapy), flow characteristics, and ventricle(s) supported. Implant suitability is determined by anatomical factors, haemodynamics, clinical eligibility criteria, and INTERMACS profiles [24, 32, 95, 97] Left ventricular assist devices (LVAD) comprise the majority of implanted devices, and their use is divided into acute (hours to weeks) and chronic (months to years).[24, 95, 97]

In recent years, survival after implantation of the latest-generation, continuous-flow, durable LVADs has improved, with one-year survival rates exceeding 80% after 2010 (median survival of 4.55 years vs 3.88 years before 2010).[95, 97-99] This progress is largely attributed to significant advancements in device engineering, such as the transition to hybrid-levitation centrifugal-flow pumps, as well as improvements in patient selection and clinical care (**Figure 15**).[98] Although tethered to external power supplies posing limitations, LVADs enhance functional capacity and quality of life in patients with advanced HF.[99-101] While these clinical benefits are significant, they are offset by increased risks of neurological events, infection, bleeding, and pump malfunction. Moreover, the highest mortality rates still occur within the first three months following implantation.[95, 98] Preoperative risk factors associated with worse outcomes include the presence of secondary pulmonary hypertension, elevated central venous pressure, frailty, and/or coagulopathy.[102-104]

Heart transplantation

While mechanical circulatory support systems offer an unparallelled technological asset in the management of advanced HF, heart transplantation (HT) remains the ultimate treatment for refractory, end-stage disease.[24, 25, 96, 97] HT leads to morbidity and mortality benefits in advanced HF, including improved functional status and health-related quality of life measures.[24, 25, 96, 105] However, the stagnant donor availability remains a key challenge amid a steadily increasing number of patients listed for HT, the latter reflected by changing transplant eligibility, allocation priorities, and improved survival of HF in recent years.[96, 105, 106] Given the substantial physical and psychosocial demands of HT, a multidisciplinary and a careful allocation process are paramount.[24, 25, 96] According to the International Society for Heart and Lung Transplantation (ISHLT) 2025 report, a substantial increase in the number of adult HT performed has been observed, exceeding 5000 annual procedures globally.[106] International reports indicate that the median survival after adult HT, between 2002 and 2009, is 12.5 years,

increasing to 14.8 years among one-year survivors, compared to less than two years for end-stage HF without advanced therapies.[24, 105] Moreover, the overall survival varies across aetiologies, with highest survival rates observed in congenital heart disease and lowest in ischaemic cardiomyopathy or retransplantation among one-year survivors. Mortality remains high during the first year after HT, with infectious complications being the leading cause of death, except for acute graft failure, which predominates in the first month after HT.[105]

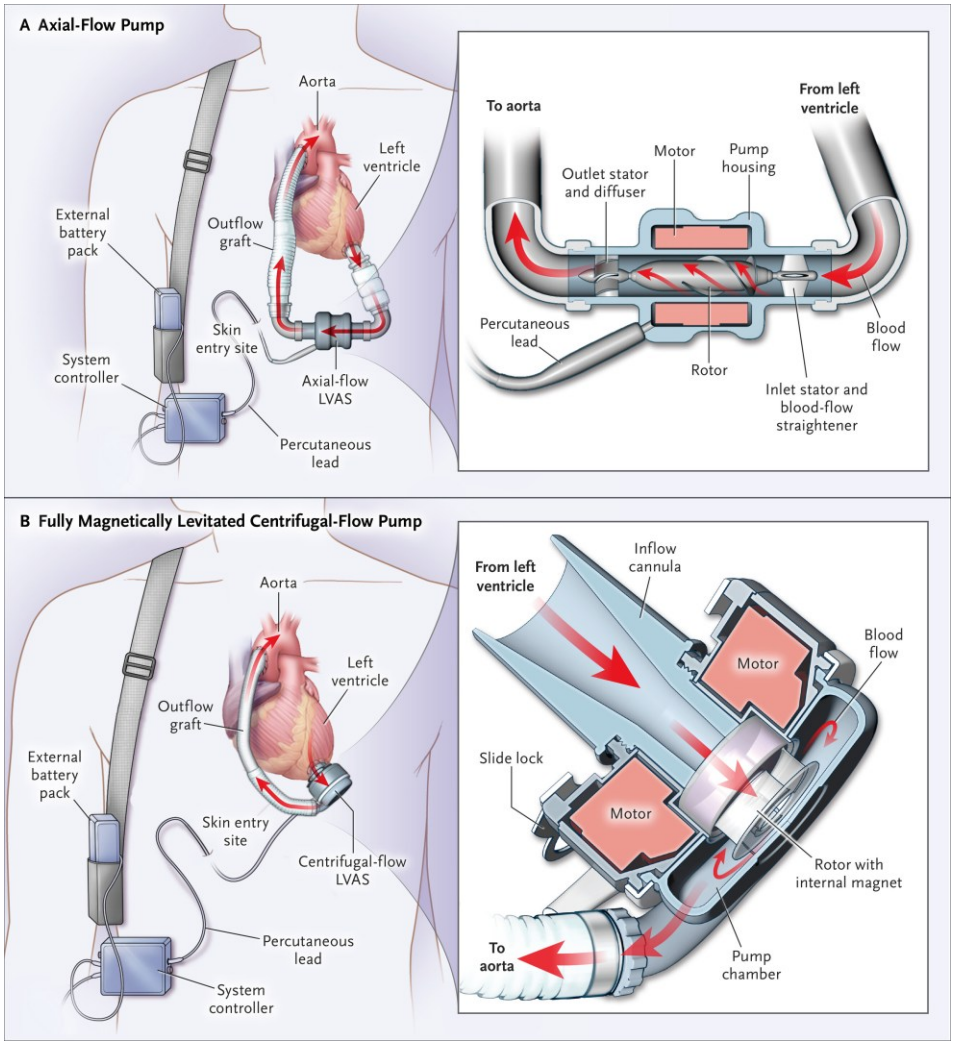

Figure 15.

Illustration of pump mechanisms (A) Axial flow pump, and (B) magnetically levitated centrifugal-flow pump, both in left ventricular assist device (LVAD) systems and the latter associated with decreased risk of thrombosis associated pump malfunction. Reproduced with permission from Mehra MR et. al. Copyright Massachusetts Medical Society.[98]

Pulmonary hypertension

Pulmonary hypertension (PH) is an umbrella term encompassing a spectrum of distinct diseases which, in their initial stages, primarily affect the cardiopulmonary system, but in more advanced forms may evolve into a systemic condition resulting in multiorgan involvement and failure.[107-109] It is a common syndrome, affecting approximately 1% of the global population, with prevalence increasing with age, exceeding 10% among individuals > 65 years of age, imposing a significant socioeconomic burden worldwide.[109-111] The World Health Organisation (WHO) classifies PH into five distinct groups based on aetiology, treatment strategies, and the predominant site of pathological vascular changes. Haemodynamically, PH represents a continuum of profiles, ranging from isolated pre-capillary- or post-capillary- forms to combined post- and pre-capillary phenotypes (**Table 1**). The diagnosis of PH requires invasive haemodynamic assessment with right heart catheterisation (RHC), indicating a mean pulmonary arterial pressure (mPAP) over 20 mmHg. Moreover, pulmonary arterial wedge pressure (PAWP), and cardiac output (CO) are required to estimate pulmonary vascular resistance (PVR), all of which are essential parameters to further characterise the nature of the underlying PH (**Figure 16**).[107, 109]

The presence of PH, in any gestalt irrespective of aetiology, constitutes a negative prognostic marker, impairing functional capacity and increasing both morbidity and mortality.[111-116] The mortality risks vary across PH groups and the sub-entities within, and is further influenced by the constellation of modifiable and non-modifiable factors, such as sex, age, comorbid burden, clinical frailty, treatment strategy, and response to therapy.[109, 111, 117, 118]

The clinical presentation of PH, characterised by a nonspecific pattern of signs and symptoms of right ventricular dysfunction, further complicates diagnosis.[109, 119, 120] These symptoms include fatigue, dyspnoea at rest and upon exertion, orthopnoea, and bendopnoea (reflecting increased filling pressures) as well as haemoptysis and (pre)syncope occurring during or shortly after physical activity.[119, 120] Other symptoms due to pulmonary artery distention may develop in later stages of the disease and may include exertional chest pain caused by dynamic compression of the left main coronary artery, and dysphonia (Ortner's syndrome) due to compressed left laryngeal recurrent nerve.[109, 121] General clinical signs indicative of right ventricular forward and backward failure may include central and peripheral cyanosis, jugular vein distention, ascites, and peripheral oedema. Additional signs suggestive of PH and its underlying aetiology may comprise systolic murmur of tricuspid regurgitation, digital clubbing, and telangiectasia.[109, 119, 120]

Table 1. Haemodynamic definitions of pulmonary hypertension (PH) and its clinical subgroups, based on right heart catheterisation, according to the 2015 and 2022 European Society of Cardiology/European Respiratory Society (ESC/ERS) guidelines

Definition	Haemodynamic characteristics 2022	Haemodynamic characteristics 2015	WHO Clinical group(s)
PH	mPAP > 20 mmHg at rest	mPAP ≥ 25 mmHg at rest	Group 1 – 5
Pre-capillary PH	mPAP > 20 mmHg at rest PAWP ≤ 15mmHg PVR > 2 WU	mPAP ≥ 25 mmHg PAWP ≤ 15 mmHg	Group 1, 3, 4, 5
Post-capillary PH	mPAP > 20 mmHg at rest PAWP > 15mmHg	mPAP ≥ 25 mmHg PAWP > 15 mmHg	Group 2, 5
Isolated post-capillary PH	mPAP > 20 mmHg at rest PAWP > 15mmHg PVR ≤ 2 WU	mPAP ≥ 25 mmHg PAWP > 15 mmHg DPG < 7 mmHg and/or PVR ≤ 3 WU	Group 2, 5
Combined post- and pre-capillary PH	mPAP > 20 mmHg at rest PAWP > 15mmHg PVR > 2 WU	mPAP ≥ 25 mmHg PAWP > 15 mmHg DPG ≥ 7 mmHg and/or PVR > 3 WU	Group 2, 5
Exercise PH	mPAP/CO slope > 3 mmHg/L/min between rest and exercise	–	–
Unclassified PH	mPAP > 20 mmHg at rest PAWP ≤ 15mmHg PVR ≤ 2 WU	–	–

Abbreviations: CO indicates cardiac output; DPG, diastolic pulmonary pressure gradient; mPAP, mean pulmonary arterial pressure; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; WU, Wood units; and WHO, World Health Organization. PH subgroups: **Group 1**: Pulmonary arterial hypertension, **Group 2**: PH associated with left heart disease, **Group 3:** PH associated with lung diseases and/or hypoxia, **Group 4**: PH associated with pulmonary artery obstructions, and **Group 5:** PH with unclear and/or multifactorial mechanisms.[109, 110]

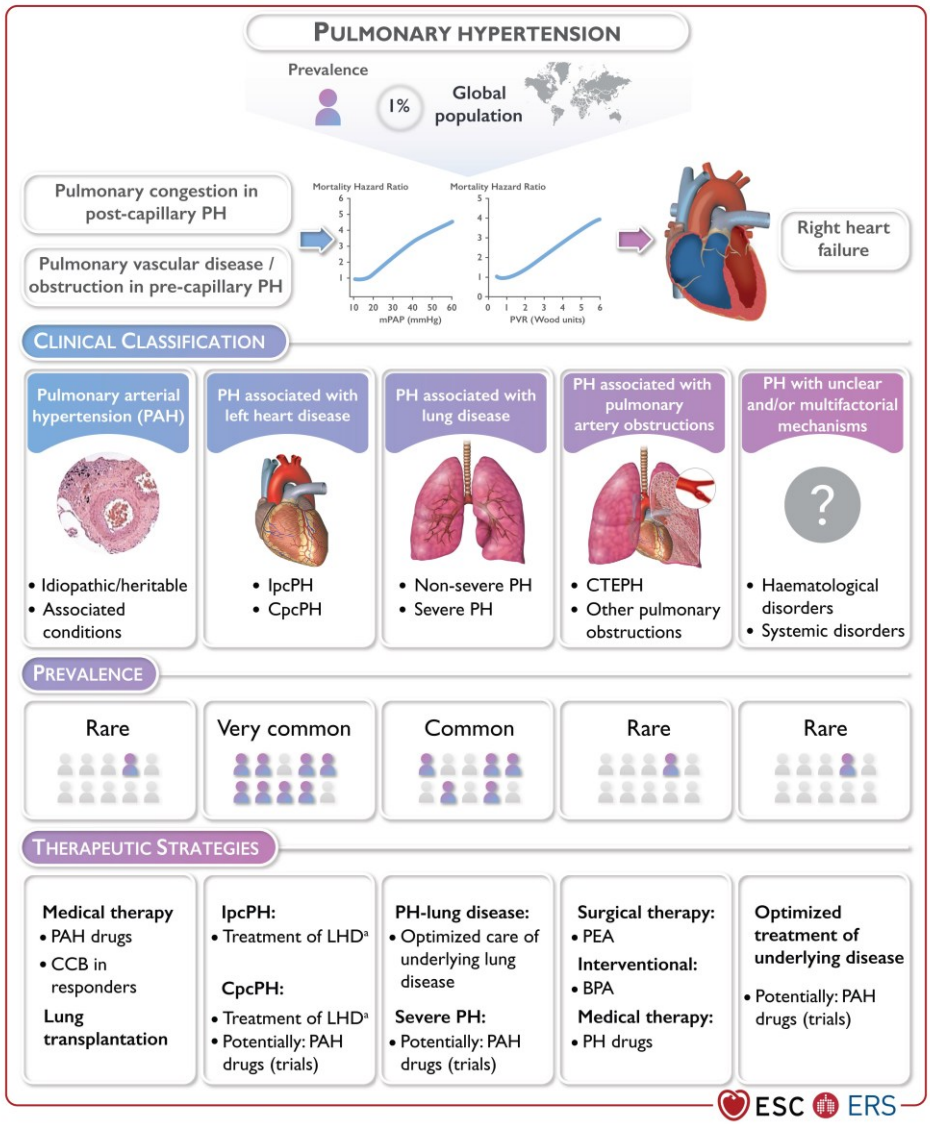

Figure 16.

Overview of pulmonary hypertension (PH) and its clinical subgroups in relation to prevalence and therapeutic strategies. Under clinical classification, The World Health Organization PH groups 1 to 5, from left to right, are displayed. Abbreviations: BPA indicates balloon pulmonary angioplasty; CCB, calcium channel blockers; CpcPH, combined post- and pre-capillary PH; IpcPH, isolated post-capillary PH; LHD, left heart disease; and PEA, pulmonary endarterectomy. Reproduced with permission (license: 6071280823169).

Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH, WHO group 1 PH) is a progressive and obliterative vasculopathy, primarily of the small pulmonary arteries, affecting all components of the pulmonary vascular wall.[122] Although incompletely understood, the development and progression of PAH are driven by an intricate interplay of dysregulated pathways, including inflammation, immunomodulation, angiogenesis, and metabolism.[108, 123] These promote pulmonary vascular obliteration and remodelling, characterised by endothelial dysfunction, vasoconstriction, pulmonary vascular rarefaction, perivascular inflammation, abnormal muscularisation, and excessive fibrosis, the latter resulting in less compliant pulmonary arteries. These changes result in increased PVR and pre-capillary PH, leading to progressive maladaptive RV remodelling and failure, functional decline and premature death.[108, 122, 123]

PAH is a rare condition, with an annual incidence of approximately 5.8 cases/million, and a prevalence of 48 – 55 cases/million inhabitants, in developed nations.[124] These estimates are consistent with epidemiological data from the national Swedish PAH registry (SPAHR), reporting an annual incidence of 8 cases/million and a prevalence of 49 per million inhabitants.[125] In developed countries, idiopathic PAH (IPAH) accounts for the majority of PAH cases (50 – 60%), followed by connective tissue disease associated PAH (CTD-APAH) including those with systemic sclerosis (15 – 30%), and congenital heart disease associated PAH (CHD-APAH, 10 – 23%).[126-128] A significant shift of PAH epidemiology has occurred over the past 40 years, denoted by a substantial increase in the number of multimorbid, elderly, and female patients with PAH compared to the young, otherwise healthy female PAH stereotype in the 1980s.[119, 125, 127, 129-132]

Before treatment was available, the median survival of newly diagnosed adults with PAH was 2.8 years, and as low as 11.8 months in Raynaud's phenomenon associated PAH.[133] Despite improved survival since the introduction of pulmonary vasodilators and improved treatment strategies, the mortality rates remain high.[125-127, 129-131, 133, 134] In Sweden, among incident patients with PAH diagnosed between 2015 and 2023, the overall 1-, 3- and 5- year survival rates are 87%, 67% and 52%, respectively.[134] The mortality rates vary among different PAH aetiologies and is lowest among long term responders to calcium channel blockers who form a limited portion (< 10%) of patients with PAH.[135, 136] Among the larger PAH sub-groups, CHD-APAH has the lowest mortality rates, contrary to CTD-APAH, in which the mortality remains the highest.[125-127, 129-131, 134]

Delay in diagnosis and management

The clinical presentation of PAH is characterised by nonspecific symptoms such as fatigue, exertional dyspnoea, and functional decline. Rather than PAH, these symptoms often reflect more common cardiopulmonary conditions including asthma, chronic obstructive pulmonary disease, atrial fibrillation, or left HF.[119, 127, 137, 138] Most of these patients, including those with PAH initially seek primary care (> 70%), often undergoing between three and ten consultations before referral to a PH specialist centre for diagnosis.[138-142]

Over the past four decades, one of the most striking observations is that the time from symptom onset to diagnostic RHC has not significantly improved with a median ranging between 1.13 and 3.66 years, compared to the median of 1.27 years in the 1980s (**Table 2**).[119, 140, 143-145] It is also estimated that 20 – 35% of the patients still wait > 2 years for PAH diagnosis, and misdiagnosis occur in up to 41%, the latter associated with longer diagnostic intervals.[142, 144, 145] Moreover, 50 – 83% of the patients are still in functional class III and IV at diagnosis.[142, 144, 146] Longer diagnostic times have also been associated with higher mortality rates independent of age, gender, and PAH subtype, and a two-year diagnostic delay was associated with an 11% increased risk of mortality, rising to 29% after a five-year delay.[144, 145] Not only do these numbers reflect the lack of PAH awareness among physicians, but also the nonspecific nature of symptoms, which are more commonly, and more likely, attributed to other cardiopulmonary diseases, underscoring that dyspnoea can be a difficult symptom to evaluate.[137, 138]

While the PAH diagnostic algorithms in the 2022 ESC/ European Respiratory Society (ERS) PH guidelines and the proceedings of the 7th World Symposium on Pulmonary Hypertension (WSPH) emphasise the importance of a multimodal and pragmatic approach in PAH diagnosis, primary care physicians may benefit from a broader and more structured framework.[107, 109, 137] Such approach should address not only the cardiopulmonary aspects, but also the multidimensional nature and the totality of dyspnoea, to ultimately reduce the diagnostic delay in PAH. Hence, as an effort to raise awareness, reduce diagnostic delay, and promote timely referral to PH centres, we developed a complementary algorithm for the structured evaluation of unclear dyspnoea (**paper VII**). The algorithm, a multistep approach, has been promoted nationally and internationally to optimise the diagnostic process based on available resources and to ensure well-founded referrals tailored to the capacity of tertiary PH expert centres.[137, 138]

Step 1 should be initiated independently of where the patient is in the health care system and include a comprehensive medical history with a thorough physical examination with assessment of vital parameters to guide and direct the further evaluation of dyspnoea. The overall aim should be directed towards finding and ruling out common causes of dyspnoea, primarily cardiopulmonary aetiologies based on the substantial number of differential diagnoses. Depending on the findings in step 1, more targeted tests can be performed according to step 2, whereafter referral to specialized PH centres (step 3), may be performed (**paper VII**, **Figure 17**).

Table 2. Time from symptom onset to PAH diagnosis and factors associated with prolonged diagnostic times

Period	First author (Registry/Country)	Number of participants	Delay mean (years)	Delay median (years)	Increasing delay factors	WHO-FC (III-IV)
1981 – 1985	Rich et al. (NIH)[119]	187	2.03	1.27	NA	71%
2002 – 2003	Humbert et al. (France)[128]	674	2.25	NA	NA	75%
2004 – 2006	Wilkens et al. (Germany)[139]	142	2.3	NA	NA	54%
2006 – 2007	Brown LM et al. (USA /REVEAL)[145]	2493	2.84 (21% > 2 years)	1.13	Age < 36 years 6MWD < 250m OSAS COPD MRAP < 10 mmHg PVR < 10 WU	73.6%
2007 – 2008	Strange et al. (Australian PH registry)[140]	32	3.92 (total)	3.66	Older age Multiple doctor visits	6%
2004 – 2017	Khou et al. (Australian and New Zealand PH registry)[144]	2044	2.5 (35% > 2 years)	1.2	Age CHD-PAH OSAS Peripheral vascular disease	82%
2018	Kopeć et al. (POLISH registry)[146]	970	1.86	NA	NA	83%
2019 – 2020	Small et al. (France, Germany, Italy, Spain, UK, Japan, USA)[142]	1152 patients 378 doctors	1.41 PP 1.15 DD	NA	Multiple doctor visits	49.9%

Abbreviations: 6MWD indicates six-minute walk distance; CHD-APAH, congenital heart disease-associated pulmonary arterial hypertension; COPD, chronic obstructive pulmonary disease; DD, doctors' delay; MRAP, mean right atrial pressure; NIH, National Institutes of Health; OSAS, obstructive sleep apnoea syndrome; NA, not available; PP, patients' delay; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; PVR, pulmonary vascular resistance, REVEAL, Registry to Evaluate Early and Long-Term PAH Disease Management; WHO-FC, World Health Organization functional class; and WU, Wood units.

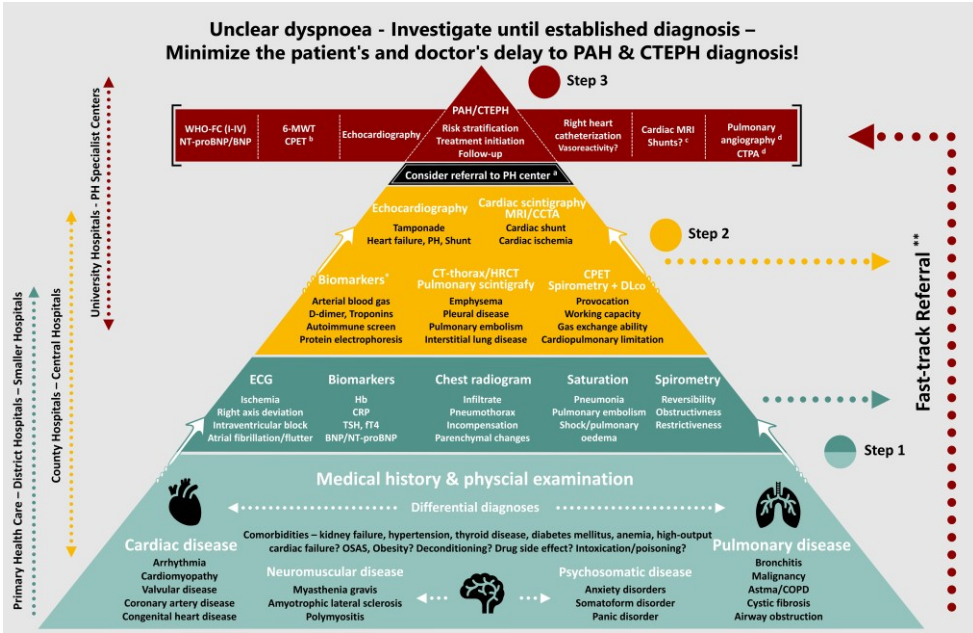

Figure 17. Multidimentional algorithm for structured evaluation of unclear dyspnoea.
The investigation of unexplained dyspnoea should follow a structured, multistep approach (steps 1 to 3) to help narrow down the broad range of possible differential diagnoses. [a] If pulmonary arterial hypertension (PAH), chronic thromboembolic pulmonary hypertension (CTEPH), or an unclear aetiology is suspected after completing steps 1 and 2 of the evaluation. [b] Cardiopulmonary exercise testing (CPET) is not routinely performed, but provides complementary prognostic information to the 6-minute walk test (6MWT) and aids in identifying whether dyspnoea is primarily of cardiac or pulmonary origin. [c] Cardiac magnetic resonance imaging (MRI), combined with sequential oxygen saturation measurement during right heart catheterisation (RHC) is used to further characterise the shunts. [d] Pulmonary angiography and/or computed tomography pulmonary angiography (CTPA) are performed in cases of suspected/imaging indicated thromboembolic pulmonary arterial disease. * Includes the use of additional disease specific biomarkers, such as sweat testing for cystic fibrosis, differential blood count, tissue biopsy, and genetic evaluation. This additionally may include the future use of novel cardiopulmonary biomarkers for dyspnoea as they emerge. ** Fast-track referral is recommended in cases with a high likelihood or suspicion of PAH or CTEPH, as outlined in the 2022 European Society of Cardiology and European Respiratory Society (ESC/ERS) PH guidelines algorithm.[109] Abbreviations: BNP indicates b-type natriuretic peptide; CCTA, coronary computed tomography angiography; COPD, chronic obstructive pulmonary disease; DLco, pulmonary diffusing capacity of carbon monoxide; NT-proBNP, N-terminal pro BNP; and OSAS, obstructive sleep apnoea syndrome. Adapted from Ahmed S et al (**paper VII**).[137]

Pulmonary hypertension associated with left heart disease

A common complication of left heart disease, specifically left HF, is post-capillary PH (Group 2, PH associated with left heart disease (PH-LHD)), accounting for 65 – 80% of all cases of PH.[111] In left HF, the structural and functional alterations may be accompanied by a progressive LV dysfunction, loss of left atrial compliance, and exercise amplified mitral regurgitation. This is followed by uncoupling of the

ventricular-arterial unit, and haemodynamically backward transmission of elevated left-sided filling pressures, which if sustained may progress to isolated post-capillary PH. Over time, a combined post- and pre-capillary PH phenotype may develop, with pulmonary vascular remodelling and right ventricular dysfunction, further worsening prognosis.[147, 148] Herein, the vascular changes occur in the small-to-medium-sized veins and capillaries.[108] Venous arterialisation, reflected by increased intimal thickness, may be followed by both dynamic and structural obliteration of pre-capillary arterioles, the latter characterised by medial- and intimal thickening.[108, 149] Endothelial dysfunction, vasoconstriction, and capillary stress failure are believed to play central roles in this process.[108, 149, 150] Elevated mPAP, PAWP, and PVR, along with reduced pulmonary arterial compliance (PAC), among other haemodynamic parameters, are predictors of poor survival in PH-LHD.[147]

Although poorly characterised, PH (assessed by either RHC or echocardiography) is estimated to affect 36 – 83% of patients with HFpEF and 40 –75% of those with HFrEF in developed countries.[147] Of those, combined post- and pre-capillary PH phenotype may develop in 20 – 30 %, further increasing mortality.[111] Other common aetiologies include severe aortic stenosis, of which 50 – 70% develop PH, increasing the mortality rates by more than a twofold.[111, 151] Likewise, in developing countries, rheumatic heart disease with mitral valve stenosis constitutes a major cause of LHD, in which echocardiographic signs suggestive of PH are observed in 29 – 53% of all cases.[152-154] It is estimated that there is at least 3 to 4 million individuals with rheumatic heart disease and PH, a phenotype associated with higher rates of adverse outcomes.[111, 155] In symptomatic patients with severe mitral stenosis, PVR fails to normalise in approximately 40% despite successful mitral valve replacement or percutaneous balloon mitral valvuloplasty.[151, 155, 156]

Apart from optimising the management of left HF and comorbidities, as well as treating potentially reversible causes such as valvopathies, pulmonary vasodilators approved for PAH are not recommended in PH-LHD, as they may result in further clinical deterioration and increased mortality[109, 157-160] In a subset of patients with advanced HF and PH, vasodilator-resistant elevation of PVR (> 5 Wood units) may be present. This "fixed" increase in PVR constitutes a relative contraindication for HT due to the high risk of acute right ventricular graft failure post-transplantation.[96, 109, 161, 162] These patients are often treated with an LVAD to reduce PVR and enable HT.[96, 109, 162]

Diagnostic challenges between PAH and HFpEF with PH

Distinguishing HFpEF with PH (HFpEF-PH) from PAH can be challenging due to overlapping clinical features. Both conditions may present with similar symptoms, echocardiographic findings, right ventricular dysfunction, and invasive haemodynamics, often with borderline PAWP.[50, 109, 151] The shifting demographic profile in PAH (characterised by older age, female sex, obesity, systemic hypertension, and atrial fibrillation) further adds to the diagnostic complexity, as these features are traditionally associated with HFpEF.[109, 111, 147, 151] The clinical distinction is of utmost importance as treatment of HFpEF-PH with pulmonary vasodilators may be detrimental.[109, 157-160] Furthermore, this process may delay appropriate recognition of PAH, and thus timely initiation of PAH-specific therapy, both of which are crucial to reduce morbidity and mortality.[137, 163]

A variety of methods have been proposed to help identify patients with borderline resting PAWP and occult diastolic dysfunction to better distinguish HFpEF-PH from PAH.[151, 157, 160, 164] While exercise RHC is considered the optimal method for identifying occult diastolic dysfunction, its need for specialised expertise, high cost, time-consuming nature, reliance on patient cooperation, and limited availability restrict its routine use in clinical practice.[24, 151, 164] Conversely, other methods, such as pre-test probability scoring, passive leg raise testing, fluid challenge, and vasoreactivity testing during RHC have been proposed as more feasible alternatives. However, the protocols for these procedures vary considerably, and the procedures themselves may yield conflicting results. Moreover, the evidence supporting their utility in distinguishing HFpEF-PH from PAH remains limited, and most methods lack adequate short- and long-term, prospective validation.[24, 109, 151, 160, 163]

Circulating biomarkers

To promote clarity in communication, standardise nomenclature, and facilitate the clinical implementation of scientific discoveries, the U.S. Food and Drug Administration (FDA) and the NIH Biomarker Working Group established the Biomarkers, EndpointS, and other Tools (BEST) resource. It describes a biomarker as "a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions". A biomarker is, however, not a clinical outcome assessment, which is how an individual functions, feels, or survives". The BEST resource further emphasises the various classes of biomarkers in relation to their clinical significance (**Table 3, Figure 18**).[165]

Several mechanistic pathways have been identified in the pathobiology of HF and PH, including dysregulated angiogenic and growth factor signalling, altered metabolism, and aberrant inflammatory and immunomodulatory pathways, many of which overlap with

mechanisms recognised in tyrosine kinase signalling and tumour biology.[40, 42, 48, 50, 108, 122, 166-168]

Table 3. Biomarker classes and their defintions according to Biomarkers, EndpointS, and other Tools resource [165]

Biomarker class	Definition
Susceptibility/risk biomarker	"A biomarker that indicates the potential for developing a disease or medical condition in an individual who does not currently have clinically apparent disease or the medical condition"
Diagnostic biomarker	"A biomarker used to detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease"
Monitoring biomarker	"A biomarker measured repeatedly for assessing status of a disease or medical condition or for evidence of exposure to (or effect of) a medical product or an environmental agent"
Prognostic biomarker	"A biomarker used to identify likelihood of a clinical event, disease recurrence, or progression in patients who have the disease or medical condition of interest"
Predictive biomarker	"A biomarker used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent"
Pharmacodynamic/response biomarker	"A biomarker used to show that a biological response, potentially beneficial or harmful, has occurred in an individual who has been exposed to a medical product or an environmental agent"
Safety biomarker	"A biomarker measured before or after an exposure to a medical product or an environmental agent to indicate the likelihood, presence, or extent of toxicity as an adverse effect"

Novel biomarkers are emerging in the fields of HF, PH and transplantation, and may complement traditional clinical assessments, thereby aiding in clinical decision-making through facilitating diagnosis, monitoring, prognostication and identification of patients who require more extensive- or are in need of invasive evaluation.[16, 45, 169, 170] These biomarkers can provide additional insight into haemodynamic status and enable earlier detection of complications, such as transplant rejection or haemodynamic deterioration.[45, 169, 170] Beyond their value as non-invasive clinical tools, they may also increase our understanding of disease pathobiology through more precise phenotypic characterisation, supporting the development of targeted interventions and novel therapies to reduce morbidity and mortality.[25, 109]

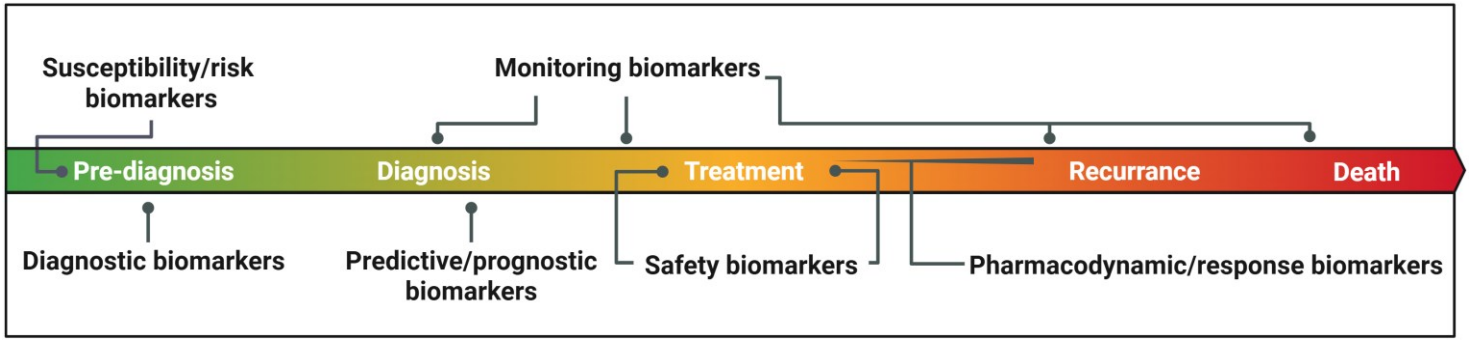

Figure 18.
The clinical use of different biomarkers classes in relation to the natural course of disease. The clinical definition of the different biomarker classes are listed in **Table 3**.[165] Created in https://BioRender.com.

Aims

Biomarkers can facilitate diagnosis, monitoring, prognosis and identification of at-risk patients requiring invasive or more urgent care. This may involve earlier PH diagnosis and differentiation to minimise diagnostic delays, improved monitoring, and earlier detection of haemodynamic deterioration in advanced HF and after transplantation.[45, 169, 170]

Therefore, the overall focus of the present thesis was to investigate plasma tyrosine kinase- and tumour-related proteins as biomarkers to facilitate non-invasive monitoring, diagnosis and prognosis of left HF and PH. In addition, the thesis aimed to optimise the clinical evaluation of dyspnoea in which novel biomarkers can be incorporated to minimise diagnostic delays in HF and PH.

Papers I – II

Papers I – II aimed to investigate the relative plasma levels of proteins belonging to receptor tyrosine kinase- (**paper I**) and tumour related signalling (**paper II**) in order to identify potential biomarkers related to advanced HF, PH and haemodynamics, before and one-year after HT.

Paper III

Paper III aimed to evaluate the absolute plasma dynamics of a panel of inflammatory and vascular proteins related to tyrosine kinase signalling, and their associations with invasive haemodynamic measurements in advanced HF, prior to- and at multiple time points during the first year after HF.

Papers IV – V

Papers IV – V aimed to investigate the clinical ability of tumour and metabolism related proteins in differentiating HFpEF-PH from PAH (**paper IV**) and in predicting prognosis in patients with LHF-PH and/or HFpEF-PH (**paper V**).

Materials and methods

Lund Cardio Pulmonary Registry

All papers in the present thesis are based on data from adults (≥18 years) enrolled in Lund Cardio Pulmonary Registry (LCPR), a local cohort of the Hemodynamic Lab, Skåne University Hospital, Lund, Sweden. LCPR encompasses clinical data and prospectively collected blood samples from healthy individuals (assessed clinically and non-invasively at enrolment) and from patients evaluated for unexplained dyspnoea, PH or HF, before and after LVAD implantation, heart- or lung transplantation. The included controls were self-declared as healthy, devoid from atrial fibrillation, ischaemic heart disease, stroke, systemic hypertension or diabetes mellitus. The registry is a blood cohort in Region Skåne's biobank, and was established by Göran Rådegran in 2011.

Blood collection, sample retrieval and protein analysis

Non-fasting venous blood samples were obtained from patients' introducers placed predominantly in the internal jugular veins during routine RHC. Peripheral venous blood, primarily from the antecubital area, was collected from healthy controls at enrolment. As per protocol, the blood samples were placed in ethylenediaminetetraacetic acid tubes, centrifuged at 2000g for 10 min at 20°C, and stored at -80°C in the LCPR biobank.

The present studies were based on venous blood samples collected between October 2011 and March 2017, retrieved for protein analyses either using proximity (**papers I, II, IV,** and **V**) or multiplex sandwich immunoassays (**paper III**) by using commercially available kits at time of analyses. Briefly, proximity extension assay is a high-throughput technique that utilises protein-specific antibody pairs conjugated to complementary DNA oligonucleotides. Upon pairwise antibody-protein binding, the DNA oligonucleotides are brought into proximity, hybridise and form a unique, protein-specific DNA sequence, which is subsequently amplified and quantified using microfluidic quantitative PCR. Attained protein levels are expressed in Olink's arbitrary units (AU) as Normalized Protein eXpression (NPX) values in Log_2, which directly correspond to the inverted C_t values (the number of cycles at which the fluorescence

threshold level is surpassed). All NPX values were linearised using $2^{(\log 2\ NPX)}$ to facilitate data interpretation prior to statistical analyses. Proteins were analysed using the Proseek Multiplex Cardiovascular II, III, and Oncology II 96-plex immunoassay panels (Olink Proteomics, Uppsala, Sweden), with each panel analysed on a separate plate.[171] Proteins were categorised according to biological mechanisms, including extracellular matrix remodelling, tyrosine kinase signalling, metabolism, and tumour biology. Each sample (well) was supplemented with four internal controls, whereas inter-plate controls and negative controls were included in triplicate on every plate. This was done for quality control, to normalise inter-plate/run variations, and determine the limit of detection. In **paper III**, multiplex sandwich immunoassays (Meso Scale Discovery, Rockville, MD) were used to quantify the absolute plasma protein concentrations, which is an ELISA based method utilising electrochemiluminescence detection, allowing for simultaneous quantification of multiple proteins in a single sample. Each protein was measured in duplicates and averaged. Values with a coefficient of variation ≥ 20% were excluded.

Ethical considerations

All studies were conducted in concordance with the ethical principles outlined in the declarations of Helsinki and Istanbul. Informed written consents were acquired prior to enrolment in LCPR and blood sample collection. Ethical approval was obtained by the local ethical board in Lund, Sweden. Ethical approval numbers Dnr: 2010/114, 2010/442, 2011/368, 2011/777, 2014/92 and 2015/270.

Clinical evaluation and right heart catheterisation

All patients underwent routine clinical evaluation according to the latest guidelines at time of inclusion, at the Hemodynamic lab at Skåne University Hospital in Lund, Sweden (a regional PH and HT referral centre). LVAD implantations and HT were performed at Skåne University Hospital in Lund, Sweden.[110, 172-177] PAH diagnosis was confirmed with RHC as a pre-capillary PH, and first established after excluding other causes of pre-capillary PH (**Figure 17**). The diagnosis of HF was based on the presence of signs and symptoms of HF, elevated natriuretic peptides and objective evidence of LV dysfunction either by echocardiography or magnetic resonance imaging. HF was further classified into (LVEF ≥ 50%) or (LVEF < 50%). All patients with HF underwent RHC as part of the routine clinical evaluation, for further characterisation of underlying PH, unexplained dyspnoea, or for consideration of advanced therapies. PH and its subgroups were further characterised according to the haemodynamic thresholds outlined in the 2015 ESC/ERS PH guidelines (**Table 1**).[110]

RHCs were performed by experienced cardiologists at rest in supine position, by inserting Swan Ganz catheter (Baxter Health Care Corp, Santa Ana, CA), predominantly into the right internal jugular vein. Vasoreactivity testing was further performed in patients with elevated PVR among patients considered for HT or with PAH. Obtained variables during RHC were: mPAP, systolic- and diastolic- pulmonary arterial pressures (sPAP, dPAP), mean right atrial pressure (MRAP), PAWP, mixed venous oxygen saturation from pulmonary artery (SvO_2), and cardiac output (thermodilution). Heart rate (HR) was assessed with electrocardiogram, arterial oxygen saturation (SaO_2) was obtained predominantly from the radial arteries, and MAP was estimated non-invasively as 1/3 systolic- + 2/3 diastolic blood pressure at time of RHC. Other calculated parameters were arteriovenous oxygen difference (a-vO_2diff), cardiac index (CI) = CO/body surface area, diastolic pulmonary pressure gradient (DPG) = dPAP – PAWP, stroke volume (SV) = CO × 1000/HR, SV index (SVI) = SV/body surface area, left ventricular stroke work index (LVSWI)=(MAP − PAWP) × SVI, right ventricular stroke work index (RVSWI) = (mPAP − MRAP) × SVI, pulmonary arterial compliance (PAC) = SV/(sPAP − dPAP), PVR = (mPAP − PAWP)/CO. In cases of multiple RHCs before HT, the clinical and haemodynamic data closest to the time before HT, or prior to LVAD implantation, were used.

Renal function was estimated either with Iohexol clearance measured within a month from RHC evaluation, or with Creatinine by using the revised Lund-Malmö glomerular filtration rate estimating equation.[178] Body surface area was estimated using Du Bois and Du Bois formula ($\text{weight(kg)}^{0.425} \times \text{height(cm)}^{0.725} \times 0.007184$).[179] Additional clinical data were retrieved from the prospective LCPR cohort database.

Software use and statistical overview

Statistical analyses were conducted using R (R Core Team), RStudio (Rstudio Team), and GraphPad Prism version for Windows (GraphPad Software, San Diego, CA). Continuous data were reported as median (inter quartile range), unless otherwise stated. Normality assumptions were assessed with histograms. Due to the dominance of non-normally distributed data, non-parametric statistics were employed as appropriate, including Mann-Whitneys U tests, Wilcoxon's signed-rank tests, and Kruskal-Wallis tests with post-hoc multiple comparisons, to assess differences in mean ranks across the study groups. Survival was described with Kaplan-Meier curves. Logistic- and Cox regression analyses were used to evaluate the diagnostic and prognostic predictive ability of proteins, and to adjust for covariables. The predictive accuracies were evaluated with Receiver Operating Characteristic (ROC) analyses, described with area under the curve (AUC). Correlation analyses were performed using Spearman's rank correlation or the repeated measures correlation method. The two-stage step-up method of false discovery rate (FDR) was used to adjust for type-I errors. P-values less than the attained FDR thresholds, or <0.05 were considered statistically significant.

Papers I – II

Study population and inclusion criteria

Papers I – II included 20 healthy controls, and 29 patients with advanced HF with or without preoperative PH, before and one-year after HT. All participants had available plasma samples at time of assessment. Of the 29 patients, one with PH at one-year post-HT and two others with missing haemodynamic data were excluded. The patients' characteristics and haemodynamic data are presented in **Table 4.**

Study setup and biomarker selection strategy

Paper I included 29 receptor tyrosine kinase associated proteins, whereas **paper II** included 48 proteins related to cancer biology (*included proteins are described in attached papers I – II*). Proteins with level dynamics reflecting both disease and healthy states (i.e., HF and subsequent recovery after HT) were selected for further analyses. These proteins were characterised by significant plasma level differences between (**i**) pre-HT and post-HT, (**ii**) pre-HT and controls, as well as (**iii**) a post-HT level change towards control levels, resembling the normalisation of level dynamics observed for N-terminal pro-B-type natriuretic peptide (NT-proBNP). Next, correlation of dynamics (post-HT – pre-HT values; Δ) were performed between proteins, NT-proBNP and improved key haemodynamic parameters after HT (mPAP, MRAP, PAWP, PVR, PAC, CI, and LVSWI), expressed by Spearman's coefficient. Correction with FDR was applied sequentially as appropriate. Proteins were ranked based on the strength and number of observed correlations (**Figures 19** and **20** for **paper I** and **II**, respectively).

Table 4. Characteristics of the patient population in papers I – II

Variable	Pre-HT (n = 26)		Post-HT (n = 26)	
	n	median (IQR)	n	median (IQR)
Female, n (%)	5 (19.2)			
Age (years)	26	50 (45 – 61)	26	52 (47 – 63)
Weight (kg)	25	80 (71 – 89)	26	78 (69 – 90)
BSA (m^2)	25	2 (1.8 – 2.1)	26	2 (1.8 – 2.1)
Creatinine (µmol/L)	25	108 (90 – 123)	26	114 (97 – 142)
eGFR (mL/min/1.73 m^2)	25	63 (55 – 71)	26	53 (43 – 72)
Atrial fibrillation, n (%)	26	13 (50)	–	–
Systemic hypertension, n (%)	26	5 (19.2)	26	3 (11.5)
Diabetes mellitus, n (%)	26	3 (11.5)	26	9 (34.6)
HF and PH classification	n (%)		n (%)	
LV ejection fraction < 50%	24 (92.3)		–	
LV ejection fraction ≥ 50%	2 (7.7)		–	
Pulmonary hypertension	19 (73.1)[A]		–	
Ipc-PH	10 (38.5)		–	

Table 4 (continued). Characteristics of the patient population in papers I – II

Variable	Pre-HT (n = 26) n (%)		Post-HT (n = 26) n (%)	
Cpc-PH	9 (34.6)		–	
HF aetiology				
Dilated Cardiomyopathy	17 (65.4)		–	
Hypertrophic Cardiomyopathy	3 (11.5)		–	
Ischaemic Cardiomyopathy	3 (11.5)		–	
Other	3 (11.5)		–	
Medications				
β-Blockers	25 (96.2)		9 (34.6)	
ACEi	11 (42.3)		–	
ARB	11 (42.3)		10 (38.5)	
MRA	22 (84.6)		3 (11.5)	
Furosemide	24 (92.3)		12 (46.2)	
Cordarone	4 (15.4)		–	
Prednisolone	1 (3.8)		25 (96.2)	
Cyclosporine	–		3 (11.5)	
Tacrolimus	–		23 (88.5)	
Mycophenolate mofetil	–		21 (80.8)	
Azathioprine	–		5 (19.2)	
Haemodynamics	n	median (IQR)	n	median (IQR)
Mean arterial pressure (mmHg)	25	82 (77 – 93)	26	102 (91 – 108)*
mPAP (mmHg)	25	29 (24 – 38)	26	14 (12 – 17)*
PAWP (mmHg)	24	20 (18 – 25)	26	7 (4 – 9.3)*
MRAP (mmHg)	25	14 (7.5 – 18)	25	3 (1 – 4)*
Heart rate (beats/min)	25	73 (69 – 82)	26	82 (73 – 89)
Cardiac output (L/min)	25	3.3 (2.6 – 4.1)	26	5.5 (5 – 6.5)*
Cardiac index (L/min/m^2)	25	1.8 (1.4 – 2.2)	26	2.8 (2.6 – 3.2)*
Stroke volume (mL/beat)	25	48 (35 – 58)	26	72 (66 – 78)*
Stroke volume index (mL/beat/m^2)	25	25 (18 – 29)	26	36 (33 – 40)*
PVR (WU)	24	2.4 (1.4 – 3.5)	26	1.4 (0.89 – 1.9)*
PAC (mL/mmHg)	25	2.2 (1.8 – 3.1)	26	5.4 (4.1 – 6.6)*

Abbreviations: ACEi indicates angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; AU, arbitrary units; BSA, body surface area; Cpc-PH, combined post-capillary and pre-capillary pulmonary hypertension; eGFR, estimated glomerular filtration rate; HT, heart transplantation; Ipc-PH, isolated post-capillary PH; IQR, inter-quartile range; LV, left ventricular; MRA, mineralocorticoid receptor antagonist; MRAP, mean right atrial pressure; mPAP, mean pulmonary arterial pressure; PAC, pulmonary arterial compliance; PAWP, pulmonary arterial wedge pressure; and PVR, pulmonary vascular resistance.

* vs Pre-HT, $p < 0.0003$, false discovery rate < 0.01.

[A] One patient suffered from severe orthopnoea, hence the unsuccessful PAWP assessment. After clinical optimisation with levosimendan and furosemide, a new haemodynamic assessment confirmed Ipc-PH.

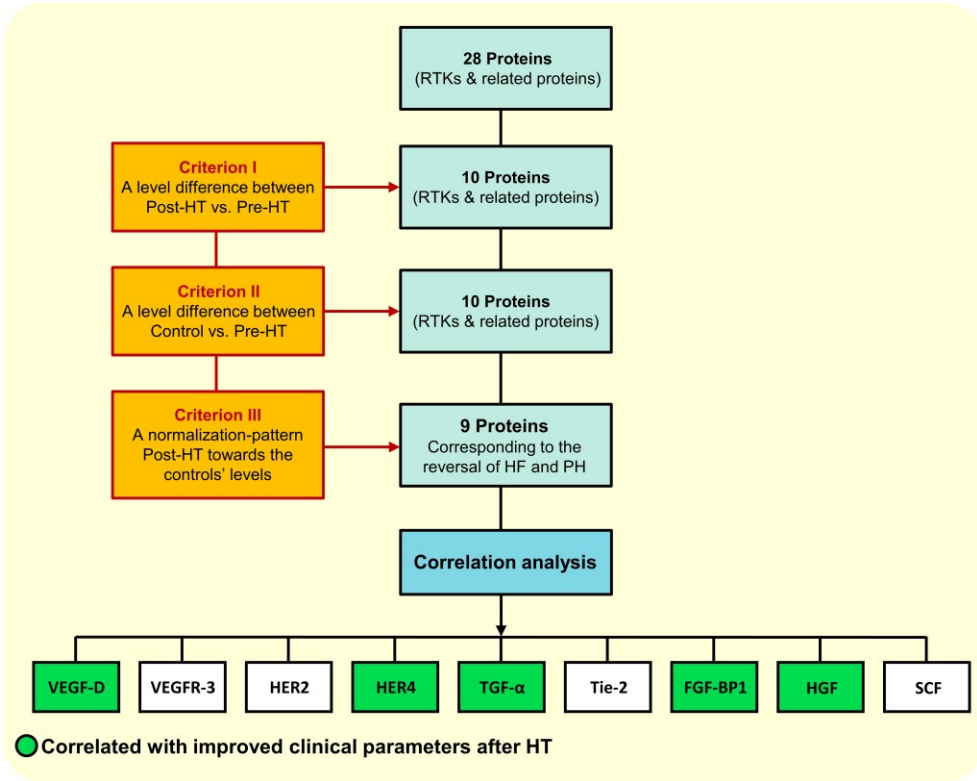

Figure 19.
Study setup, biomarker selection strategy and main results in **paper I**. Paired analyses were performed using Wilcoxon signed-rank tests, and unpaired analyses with Mann-Whitney U tests; both of which were used to assess differences in protein levels (Criteria I-III). Spearman's rank correlation analyses were performed to assess associations between proteins and variables. Abbreviations: FGF-BP1 indicates fibroblast growth factor-binding protein 1; HER, human epidermal growth factor receptor; HF, heart failure; HGF, hepatocyte growth factor; HT, heart transplantation; PH, pulmonary hypertension; Post-HT, one-year after HT; RTK, receptor tyrosine kinase; SCF, stem cell factor; TGF-α, transforming growth factor alpha; Tie-2, angiopoietin-1 receptor; VEGF-D, vascular endothelial growth factor D; and VEGFR-3, VEGF receptor 3. Adapted from **paper I** under the Creative Commons Attribution license (CC BY).

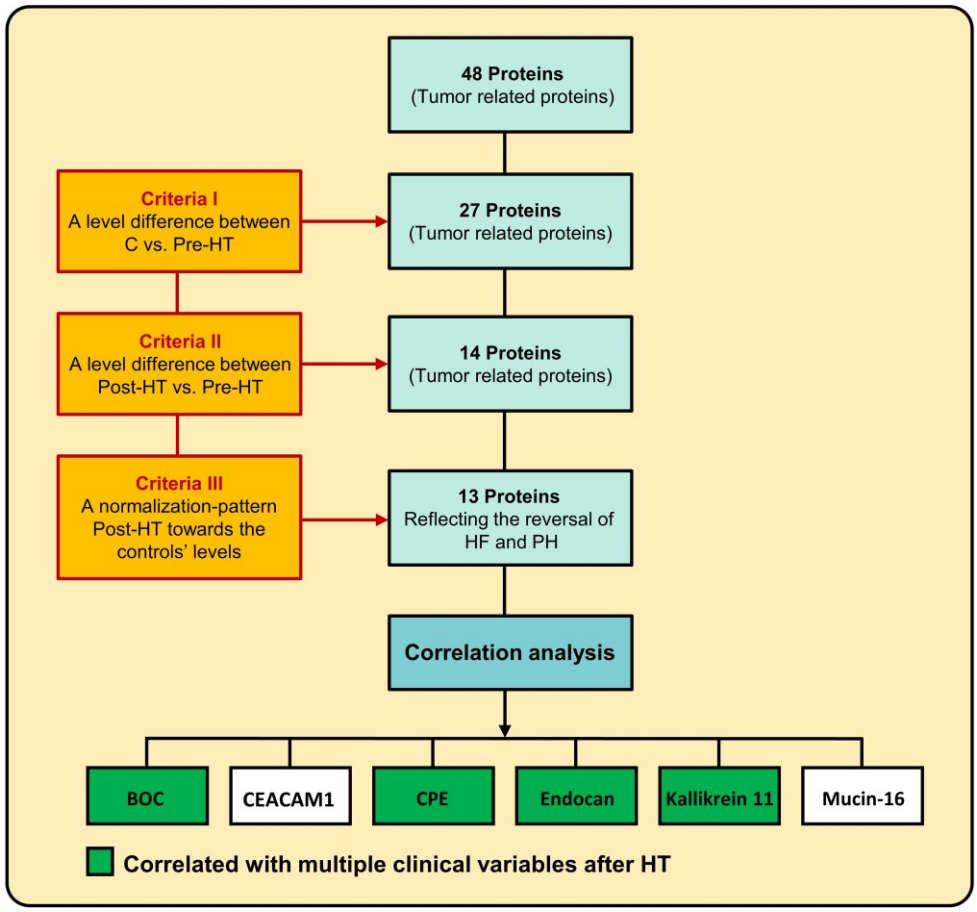

Figure 20.
Study setup, biomarker selection strategy and main results in **paper II**. To assess protein level differences between groups, Wilcoxon signed-rank tests, and Mann-Whitney U tests were used for paired and unpaired data, respectively (Criteria I-III). Spearman's rank correlation was used to examine the relationships between proteins, NT-proBNP, and haemodynamic parameters. Abbreviations: BOC indicates brother of CDO; C, control; CEACAM1, carcinoembryonic antigen-related cell adhesion molecule 1; CPE, carboxypeptidase E; HF, heart failure; HT, heart transplantation; PH pulmonary hypertension; and Post-HT; one-year after HT. Adapted from **paper II** under the Creative Commons Attribution license (CC BY).

Paper III

Study population and inclusion criteria

Paper III comprised 30 patients with advanced HF, assessed before, and at four-weeks, six-months, and one-year after HT, all with available plasma samples at time of assessment. Of the 30 patients, three with missing preoperative haemodynamics at time of blood sampling (due to implanted LVAD) and one with PH after HT were excluded. Clinical symptoms or endomyocardial biopsy findings indicative of acute rejection were absent in all patients at the times of blood sampling and haemodynamic assessments. The study population characteristics are presented in **Table 5** and **Table 6**.

Study setup and biomarker selection strategy

Paper III included nine inflammatory and vascular proteins related to tyrosine kinase signalling (*included proteins are described in the attached paper III*). Proteins showing level differences at various time points after HT compared to pre-HT levels were eligible for correlation analyses with haemodynamic parameters. After FDR correction, significant proteins were analysed with repeated measures correlations to assess the trajectory of plasma protein levels in relation to haemodynamic alterations over time. Bootstrapping was applied to estimate the confidence intervals of the correlation coefficients.

Table 5. Baseline characterises of the study population of paper III

Variable	Before heart transplantation	
	n = 26	Values
Female, n (%)	26	7 (26.9)
Age (years)	26	50.0 (42.3 – 60.0)
Age at transplantation (years)	26	50.0 (44.3 – 60.3)
BSA (m^2), median (IQR)	26	2 (1.8 – 2.2)
Left ventricular ejection fraction (%)	24	25.0 (10.0 – 27.5)
Pre-HT LVAD, n (%)	26	11 (42)
NT-proBNP, (ng/L)	21	4126 (3663 – 7306)
eGFR-creatinine (ml/min/1.73 m^2)	26	62.4 (53.7 – 71.0)
Iohexol GFR, (ml/min/1.73 m^2)	23	61 (46 – 74.5)
Atrial fibrillation, n (%)	26	7 (26.9)
Diabetes mellitus, n (%)	25	2 (8)
History of smoking, n (%)	25	7 (28)
Stroke, n (%)	26	2 (7.7)
Systemic hypertension, n (%)	26	3 (11.5)
Dilated cardiomyopathy, n (%)	26	19 (73.1)
Hypertrophic cardiomyopathy, n (%)	26	2 (7.7)
Restrictive cardiomyopathy, n (%)	26	1 (3.8)
Ischaemic cardiomyopathy, n (%)	26	3 (11.5)
Re-heart transplanted, n (%)	26	1 (3.8)

Abbreviations: BSA indicates body surface area; eGFR, estimated glomerular filtration rate; LVAD, left ventricular assist device; and NT-proBNP, N-terminal pro-B-type natriuretic peptide. Adapted from **paper III** under the Creative Commons Attribution license (CC BY).

Table 6. Haemodynamic characteristics of the study population in paper III

Variable	Before HT		4 weeks after HT		6 months after HT		1 year after HT	
	n=26	Values	n=24	Median (IQR)	n=26	Median (IQR)	n=17	Median (IQR)
PH classification								
PH-LHD, n (%)	24	19 (79.2)	–	–	–	–	–	–
Ipc-PH, n (%)	19	11 (57.9)[A]	–	–	–	–	–	–
Cpc-PH, n (%)	19	7 (36.8)	–	–	–	–	–	–
Haemodynamics		Median (IQR)						
Mean arterial pressure (mmHg)	24	83 (78 – 94)	22	93 (85 – 100)	24	103 (98 – 115)	17	105 (97 – 115)
mPAP (mmHg)	24	31 (25 – 39)	22	18 (12 – 21)	24	17 (12-19)	17	16 (13 – 21)
PAWP (mmHg)	23	20 (18 – 27)	22	9.5 (5 – 11)	24	7 (5-10)	17	6 (5-9.5)
MRAP (mmHg)	24	15 (9 – 18)	22	4.5 (3 – 6)	24	2.5 (1-4)	17	2 (0.5-4.5)
Cardiac output (liter/min)	24	3.2 (2.6 – 4)	22	5.9 (5.4 – 6.7)	24	5.5 (5.1-6)	16	5.3 (5-6.1)
Stroke volume (ml/beat)	24	45 (34 – 57)	22	71 (64 – 80)	24	74 (65-80)	16	75 (68-81)
a-vO_2diff (ml O_2/liter)	24	74 (66 – 88)	15	43 (40 – 49)	20	46 (42-49)	14	45 (39-49)
PVR (WU)	23	2.5 (2.1 – 3.5)	22	1.3 (0.96 – 1.5)	24	1.5 (1.1-1.9)	16	1.7 (1.2-2.1)
PAC (ml/mmHg)	24	2.3 (1.8 – 3.5)	21	5.3 (4 – 6.9)	24	4.7 (3.5-6.4)	16	4.9 (3.8-6.2)

Abbreviations: a-vO_2diff indicates arteriovenous oxygen difference; Cpc-PH, combined post- and pre-capillary pulmonary hypertension; Ipc-PH, isolated post-capillary PH; MRAP, mean right atrial pressure; mPAP, mean pulmonary arterial pressure; PAC, pulmonary arterial compliance; PAWP, pulmonary arterial wedge pressure; PH-LHD, PH associated with left heart disease; and PVR, pulmonary vascular resistance.

[A] One patient had severe orthopnoea during right heart catheterisation and PAWP could not be assessed. Subsequent haemodynamic assessment after clinical optimisation revealed an Ipc-PH.

Papers IV – V

Study population and inclusion criteria

Papers IV – V included 20 healthy controls, 48 patients with PAH, and 67 patients with left HF and post-capillary PH (LHF-PH) comprising 36 with HFrEF-PH and 31 with HFpEF-PH, after excluding three patients with missing haemodynamic data and two with unconfirmed diagnosis. All participants had available plasma samples at time of enrolment (controls), and at diagnostic RHC for patients. The study population is described more thoroughly in **Table 7.**

Study setup and biomarker selection strategy

Papers IV – V initially included 69 tumour- and metabolism- related proteins (*included proteins are described in attached papers IV – V*). To define proteins related to LHF and/or HFpEF-PH and to limit the number of tests performed, a sequential approach using non-parametric tests and FDR correction was employed (**Figure 21**). According to the pattern of protein levels observed between the groups, proteins were classified as either: (**i**) HFpEF-PH specific (significant level difference in Control vs HFpEF-PH and HFpEF-PH vs HFrEF-PH), or (**ii**) LHF-PH (no difference in HFrEF-PH vs HFpEF-PH, and significant difference between Controls vs HFpEF-PH and Controls vs. HFrEF-PH). Proteins expressing no difference between HFpEF-PH and Controls or present with an unclear plasma level pattern were excluded (**Figure 21**). The protein classification guided the selection of either the HFpEF-PH group (n = 31) or the pooled LHF-PH group (n = 67) for subsequent diagnostic and prognostic analyses.

Paper IV

In **paper IV**, protein levels significantly different in HFpEF-PH from PAH were assessed with ROC curves to determine protein specific thresholds and to select the three proteins with largest AUCs in identifying HFpEF-PH from PAH. Logistic regression analyses were conducted to combine and adjust for age and sex for each of the three proteins, followed by ROC analyses of the models and internal bootstrap validation of the proteins' diagnostic performances. Youden's index or closest distance to top left method, were used to determine the most optimal thresholds (yielding the highest sensitivity and specificity) for potential biomarkers and models. Biomarker level associations with NT-proBNP and key haemodynamics parameters of diagnostic value were preformed using Spearman's rank correlation (**Figure 21**).

Table 7. Characteristics of the patient population in papers IV – V

Variable	LHF-PH (n = 67)	HFpEF-PH (n = 31)	HFrEF-PH (n = 36)	PAH (n = 48)
Female, n (%)	27 (40.3)	20 (64.5)	7 (19.4)	40 (83.3)
Age (years)	63 (51 – 75)	76 (69 – 83)	54 (47.3 – 59.5)	71.5 (64 – 76)
Body surface area (m^2)	1.94 (1.81 – 2.13)	1.89 (1.72 – 2.13)	2.01 (1.89 – 2.13)	1.75 (1.59 – 1.96)
eGFR (mL/min/1.73 m^2)	53.9 (39.6 – 66.2) (n-4)	48.3 (38.4 – 63.4) (n-3)	57 (43.3 – 70.4) (n-1)	58.7 (43.4 – 68.7) (n-3)
NT-proBNP (AU)	12.97 (7.6 – 32.1)	7.6 (5.0 – 9.9)	30.2 (17.2 – 43.3)	8.8 (4.2 – 14.3)
Atrial fibrillation, n (%)	37 (56.1) (n-1)	23 (76.7) (n-1)	14 (38.9)	8 (16.7)
Diabetes mellitus, n (%)	14 (21.9) (n-3)	10 (35.7) (n-3)	4 (11.1)	12 (25)
Systemic hypertension, n (%)	27 (42.9) (n-4)	20 (74.1) (n-4)	7 (19.4)	17 (35.4)
Ischeamic heart disease, n (%)	12 (20.0) (n-7)	6 (25.0) (n-7)	6 (16.7)	7 (14.6)
Thyroid illness	5 (8.3) (n-7)	2 (8.3) (n-7)	3 (8.3)	11 (22.9)
Stroke, n (%)	10 (16.4) (n-6)	6 (24.0) (n-6)	4 (11.1)	2 (4.2)
WHO-FC, (n) 1/2/3/4	–	–	–	1/9/28/2 (n-8)
PH classification	n (%)	n (%)	n (%)	n (%)
LHF-PH	67 (100)	31 (100) A	36 (100)	–
Ipc-PH	30 (44.8)	11 (35.5)	19 (52.8) B	–
Cpc-PH	27 (40.3)	20 (64.5)	17 (47.2)	–
PAH	–	–	–	48 (100)
IPAH	–	–	–	21 (43.8)
FPAH	–	–	–	2 (4.2)
SSc-APAH	–	–	–	21 (84)
CTD-APAH	–	–	–	4 (16)
Medications	n (%)	n (%)	n (%)	n (%)
β-Blockers	58 (86.6)	23 (74.2)	35 (97.2)	16 (33.3)
ACEi	30 (44.8)	11 (35.5)	19 (52.8)	10 (20.8)
ARB	24 (35.8)	10 (32.3)	14 (38.9)	4 (8.3)
MRA	36 (53.7)	8 (25.8)	28 (77.8)	12 (25)
Furosemide	40 (59.7)	6 (19.4)	34 (94.4)	11 (22.9)

Table 7 (continued). Characteristics of the patient population in papers IV – V

Variable	LHF-PH (n = 67)	HFpEF-PH (n = 31)	HFrEF-PH (n = 36)	PAH (n = 48)
Haemodynamics	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
MAP (mmHg)	89.3 (78.7 – 99.3)	99.3 (91.3 – 105.7)	79.3 (75.5 – 88.5)	96.2 (89.4 – 103.9)
mPAP (mmHg)	34 (29 – 43)	34 (29 – 46)	34.5 (29 – 40.8)	43 (37 – 54.8)
PAWP (mmHg)	22 (18 – 26.3)	18 (17 – 23)	25 (19 – 28) $^{(n-1)}$	8 (6 – 11)
MRAP (mmHg)	13 (8 – 16)	10 (7 – 14)	14.5 (9 – 17)	7 (4 – 11)
SVI (mL/beat/m^2)	27.3 (21.6 – 33.8)	33.8 (27.8 – 42.3)	22.5 (18.3 – 27.2)	28.7 (22.6 – 34.9)
Cardiac index (L/min/m^2)	1.9 (1.5 – 2.4)	2.3 (2.1 – 2.6)	1.6 (1.4 – 1.9)	2.2 (1.8 – 2.8)
PAC (mL/mmHg)	1.8 (1.5 – 2.8)	1.8 (1.3 – 2.7)	2.0 (1.7 – 3.0)	1.1 (0.9 – 1.50)
PVR (WU)	3.4 (2.4 – 4.1) $^{(n-1)}$	3.6 (2.5 – 5.1)	3.0 (2.3 – 3.7) $^{(n-1)}$	9.5 (6.2 – 11.8)
a-vO$_2$diff (mL O$_2$/L)	61.1 (51.4 – 78.8)	53.1 (46.4 – 57.8)	77.4 (66.6 – 86.4)	53.1 (41.8 – 63.7)

Abbreviations: ACEi indicates angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; a-vO$_2$diff, arteriovenous oxygen difference; eGFR, creatinine-based estimation of glomerular filtration rate; IQR, interquartile range; MAP, mean arterial pressure; mPAP, mean pulmonary arterial pressure; MRA, mineralocorticoid receptor antagonist; MRAP, mean right atrial pressure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PAH, pulmonary arterial hypertension; IPAH, idiopathic PAH; FPAH, familiar PAH; SSc-APAH, PAH associated with systemic sclerosis; CTD-APAH, PAH associated with connective tissue disease; LHF-PH, left heart failure with post-capillary pulmonary hypertension; IpC-PH isolated post-capillary PH; CpC-PH combined post- and pre-capillary PH; HFpEF-PH/HFrEF-PH, heart failure with preserved/reduced ejection fraction with PH; PAC, pulmonary arterial compliance; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; SVI, stroke volume index; and WHO-FC, World Health Organisation functional class.

In (n–x), the superscript "x" represents the number of missing values in each group.

[A] Borderline diagnostic PAWP (15 mmHg) in five patients who subsequently underwent fluid challenge, passive leg raise test and/or multidisciplinary discussion for final diagnosis.

[B] In one patient, after an unsuccessful initial PAWP assessment due to severe orthopnoea, Ipc-PH was confirmed at a repeat right heart catheterisation following optimisation with furosemide and levosimendan.

Paper V

In **paper V**, ROC analyses were performed to assess the proteins' univariable prognostic accuracies to identify events (death or HT) vs event-free survival, and to define the optimal protein- and model specific thresholds. The proteins were ranked according to AUC, and the five with the largest areas were selected for further Kaplan analyses. Censoring was defined as the end of study-follow-up. Due to the limited number of events (n = 53), each Cox regression model was adjusted for age, sex, and the two most common comorbidities atrial fibrillation and systemic hypertension. As in **paper IV**, Spearman's rank correlation analyses were performed between biomarker levels, NT-proBNP and haemodynamic parameters. Optimal protein level thresholds were determined using either Youden's index or the shortest distance to the top-left corner of ROC curves. To evaluate differences in predictive ability, the log-rank test was applied to groups defined by dichotomised protein levels. P-values less than 0.05 were considered statistically significant (**Figure 21**).

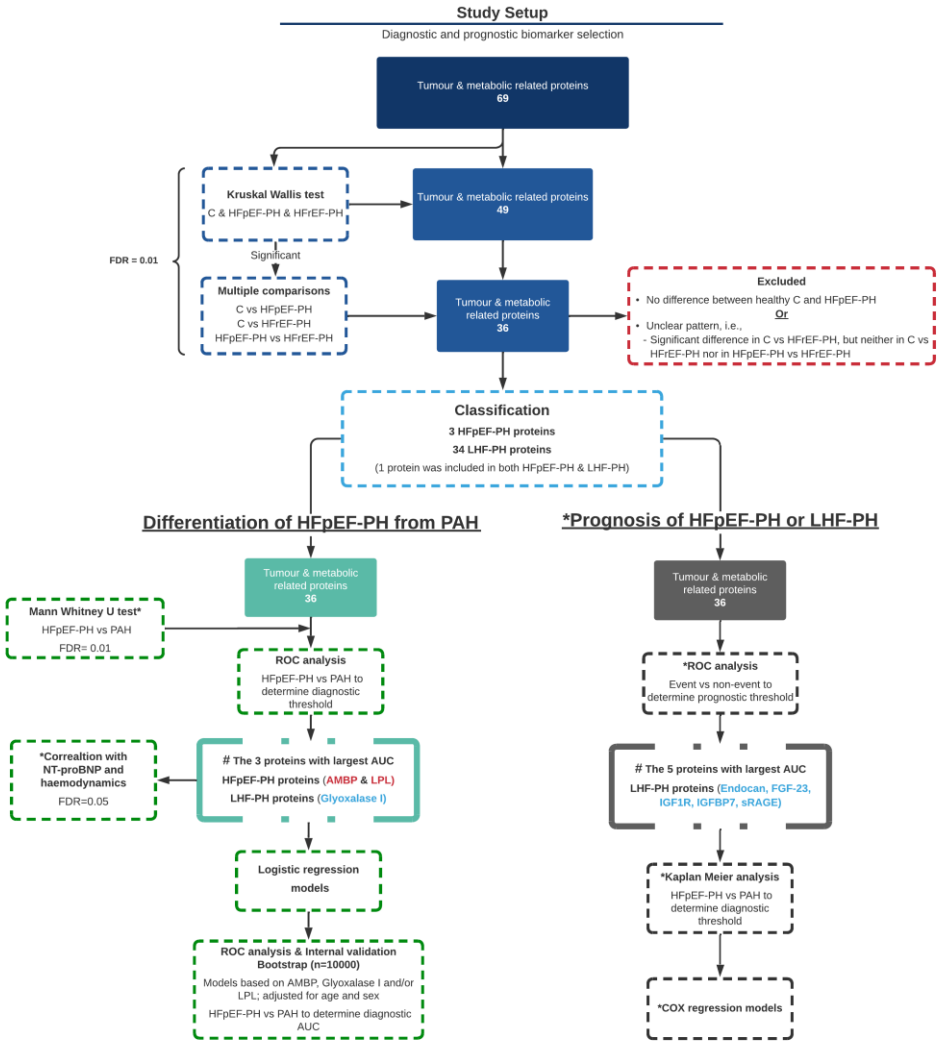

Figure 21.
Study setup, biomarker selection strategy and main results in **paper IV** (green arm) and **paper V** (grey arm). Abbreviations: AMBP indicates protein AMBP or alpha-1-microglobulin/bikunin precursor; AUC, area under the curve; C, control; FDR, false discovery rate; FGF-23, fibroblast growth factor 23; HFrEF-PH, heart failure with reduced ejection fraction and PH; HFpEF-PH, HF with preserved EF and PH; IGF1R, insulin-like growth factor 1 receptor; IGFBP7, insulin-like growth factor-binding protein 7; LHF-PH, left HF with PH; LPL, lipoprotein lipase; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PAH, pulmonary arterial hypertension; ROC, receiver operating characteristic curve; and sRAGE, soluble receptor for advanced glycation end products.* The use of either the pooled group of LHF-PH (n = 67) or HFpEF-PH (n = 31), according to the protein's classification. # The number of proteins selected with largest AUCs (three and five for the diagnostic and prognostic arms, respectively) were determined by the number of events in each arm. Adapted from **paper IV** under the Creative Commons Attribution license (CC BY).

Results

Papers I – II

Study population

The controls were 50% female (n = 10), younger at 41 (26.8 – 50.5) years, and had lower NT-proBNP levels 1.1 (1.1 – 1.2) AU, compared to the other groups. MAP 95 (88.8 – 99.8) mmHg, SaO_2 98 (97 – 98) %, and BSA 1.92 (1.75 – 1.99) m^2 were within normal ranges. Two of the controls had a history of thyroid disease. In the patient population with advanced HF, haemodynamics improved after HT, characterised by increases in MAP, CO, CI, SV, SVI, and PAC, and decreases in mPAP, PAWP, and MRAP compared to values before HT ($p < 0.0003$, FDR < 0.01, **Table 4**).

Plasma protein levels

In **paper I**, the plasma levels of vascular endothelial growth factor D (VEGF-D) and human epidermal growth factor receptor 4 (HER4) were elevated in advanced HF before HT. After HT and haemodynamic improvement, these levels decreased/normalised matching the levels of healthy controls. In **paper II**, a similar pattern was observed in the tumour related proteins endocan and brother of CDO (BOC). The plasma levels of other proteins are described in attached **papers I – II**.

Correlation analyses

The plasma level dynamics of VEGF-D, HER4, endocan and BOC (Post-HT − Pre-HT, Δ) correlated with corresponding improvements in haemodynamics and/or NT-proBNP (**Figures 22 – 25**, respectively). Of the initially included 28 tyrosine kinase related proteins (**paper I**) and 48 tumour related proteins (**paper II**), VEGF-D, HER4, endocan and BOC correlated with the largest number of and strongest with ΔNT-proBNP and/or Δhaemodynamic parameters following HT (**Figures 19 and 20**). Further subanalyses demonstrated that plasma VEGF-D and HER4 levels were also associated with baseline haemodynamic- and clinical variables ($p < 0.05$, **Table 8**). The levels of other proteins and their haemodynamic correlations are presented in the attached **papers I – II**.

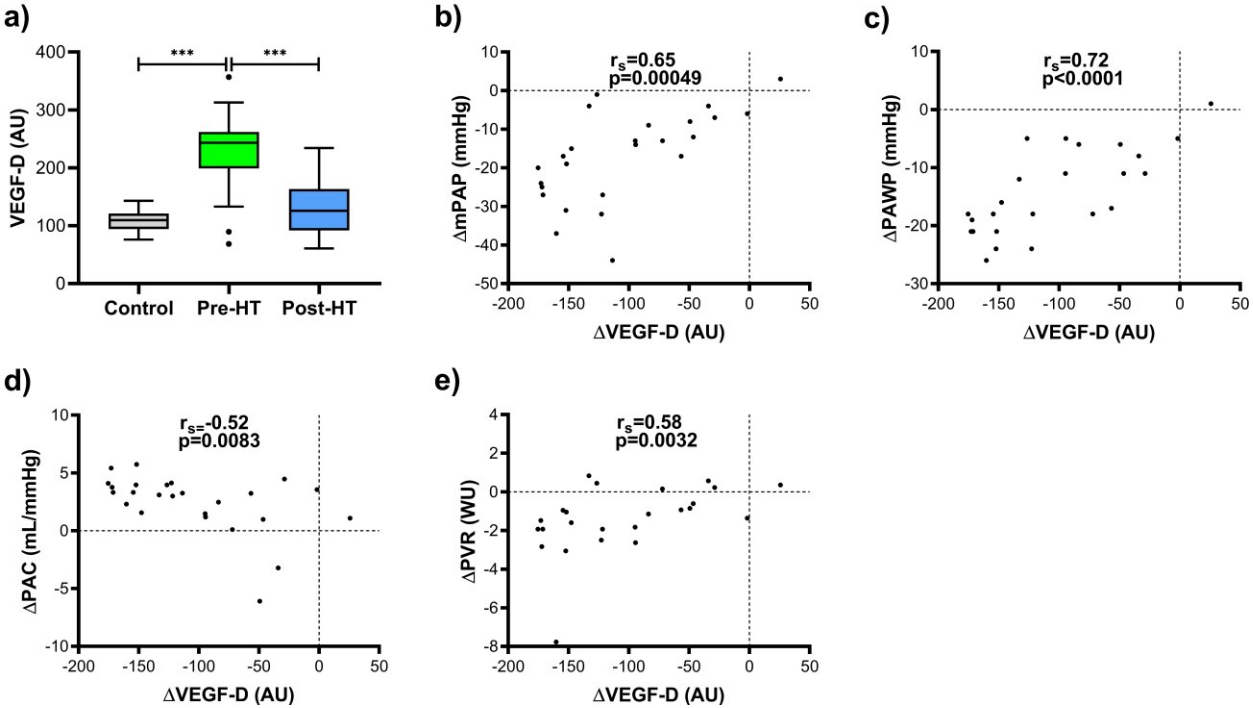

Figure 22.
Plasma levels of **(a)** vascular endothelial growth factor D (VEGF-D) in controls and in patients with advanced heart failure before and one year after heart transplantation (Pre-HT and Post-HT). Panels **(b – e)** show significant correlations between changes (Post-HT – Pre-HT values, Δ) in plasma levels and haemodynamic parameters. The decrease in VEGF-D levels after heart transplantation correlated with changes in mPAP, PAWP, PAC, and PVR. Tukey's fence method was used to define outliers. Abbreviations: AU indicates arbitrary units; mPAP, mean pulmonary arterial pressure; MRAP, mean right atrial pressure; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; and r_s, Spearman's correlation coefficient. *** $p < 0.0001$. False discovery rate < 0.01 in **(a)**, and < 0.1 in **(b – e)**. Adapted from **paper I** under the Creative Commons Attribution license (CC BY).

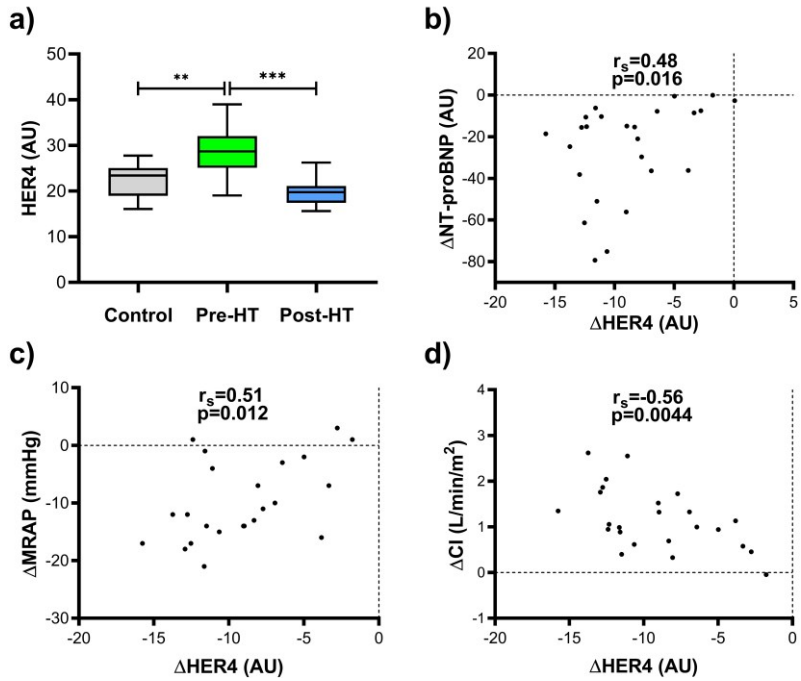

Figure 23.
Plasma levels of **(a)** human epidermal growth factor receptor 4 (HER4) in controls and in patients with advanced heart failure before and one year after heart transplantation (Pre-HT and Post-HT). Panels **(b – d)** show significant correlations between changes (Post-HT – Pre-HT values, Δ) in plasma levels and haemodynamic parameters. The decrease in HER4 levels after heart transplantation correlated with changes in NT-proBNP, MRAP, and CI. Tukey's fence method was used to define outliers. Abbreviations: AU indicates arbitrary units; CI, cardiac index; MRAP, mean right atrial pressure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and r_s, Spearman's correlation coefficient.
** p < 0.001, *** p < 0.0001. False discovery rate < 0.01 in **(a)**, and < 0.1 in **(b – d)**.
Adapted from **paper I** under the Creative Commons Attribution license (CC BY).

Table 8. Subanalysis of baseline correlations of VEGF-D and HER4 with selected haemodynamic variables

Pre-HT	n (26)	r_s (p-value)	Pre-HT	n (26)	r_s (p-value)
VEGF-D (AU) vs:			**HER4 (AU) vs:**		
mPAP (mmHg)	25	0.54 (0.0052)*	MRAP (mmHg)	24	0.35 (0.093)*
PAWP (mmHg)	24	0.42 (0.041)*	NT-proBNP (AU)	25	0.35 (0.084)*
PAC (mL/mmHg)	25	-0.69 (0.00015)*	CI (L/min/m^2)	24	-0.14 (0.51)
PVR (WU)	24	0.66 (0.00048)*			

Abbreviations: AU indicates arbitrary units; CI, cardiac index; HER4, human epidermal growth factor receptor 4; mPAP, mean pulmonary arterial pressure; MRAP, mean right atrial pressure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PAC, pulmonary arterial compliance; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; rs, Spearman's correlation coefficient; VEGF-D, vascular endothelial growth factor D; and WU, wood units. * p < 0.05. Adapted from **paper I** under the Creative Commons Attribution license (CC BY).

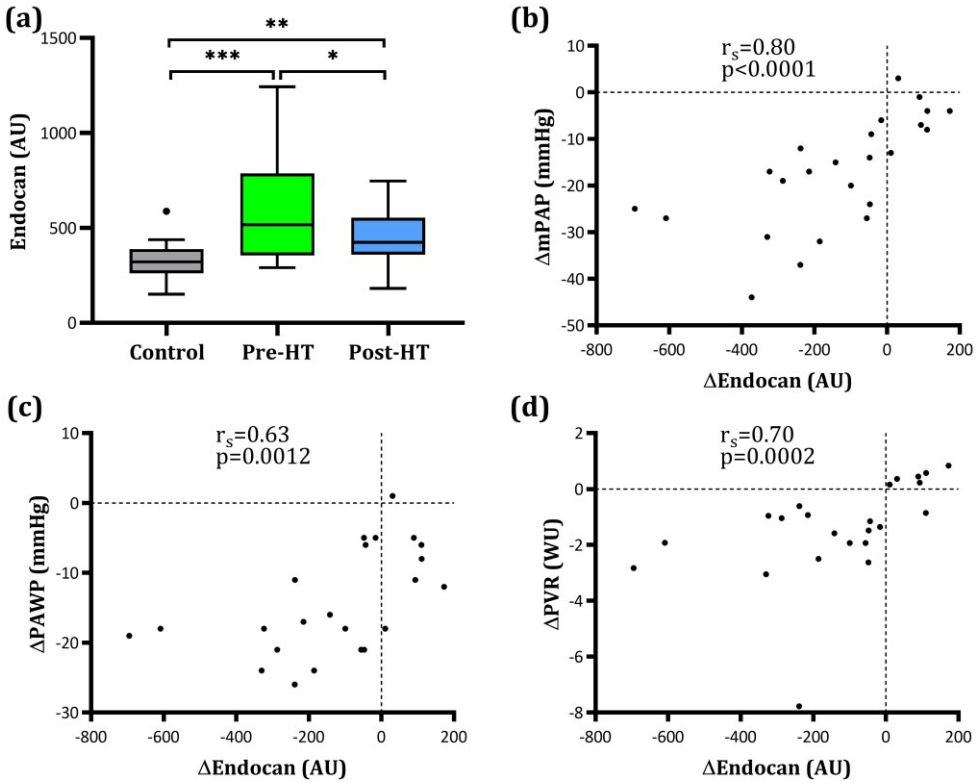

Figure 24.

Plasma levels of (**a**) Endocan in controls and in patients with advanced heart failure before and one year after heart transplantation (Pre-HT and Post-HT). Significant correlations between changes (Post-HT – Pre-HT values, Δ) in plasma levels and haemodynamic parameters are displayed in **(b – d)**. The decrease in Endocan levels after heart transplantation correlated with changes in **(a)** mPAP, **(b)** PAWP, and **(c)** PVR. Outliers were defined with Tukey's fence. Abbreviations: AU indicates arbitrary units; mPAP, mean pulmonary arterial pressure; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; and r_s, Spearman's correlation coefficient. * p <0.01; ** p < 0.001; *** p < 0.0001. False discovery rate < 0.01 in **(a)**, and < 0.1 in **(b – d)**. Adapted from **paper II** under the Creative Commons Attribution license (CC BY).

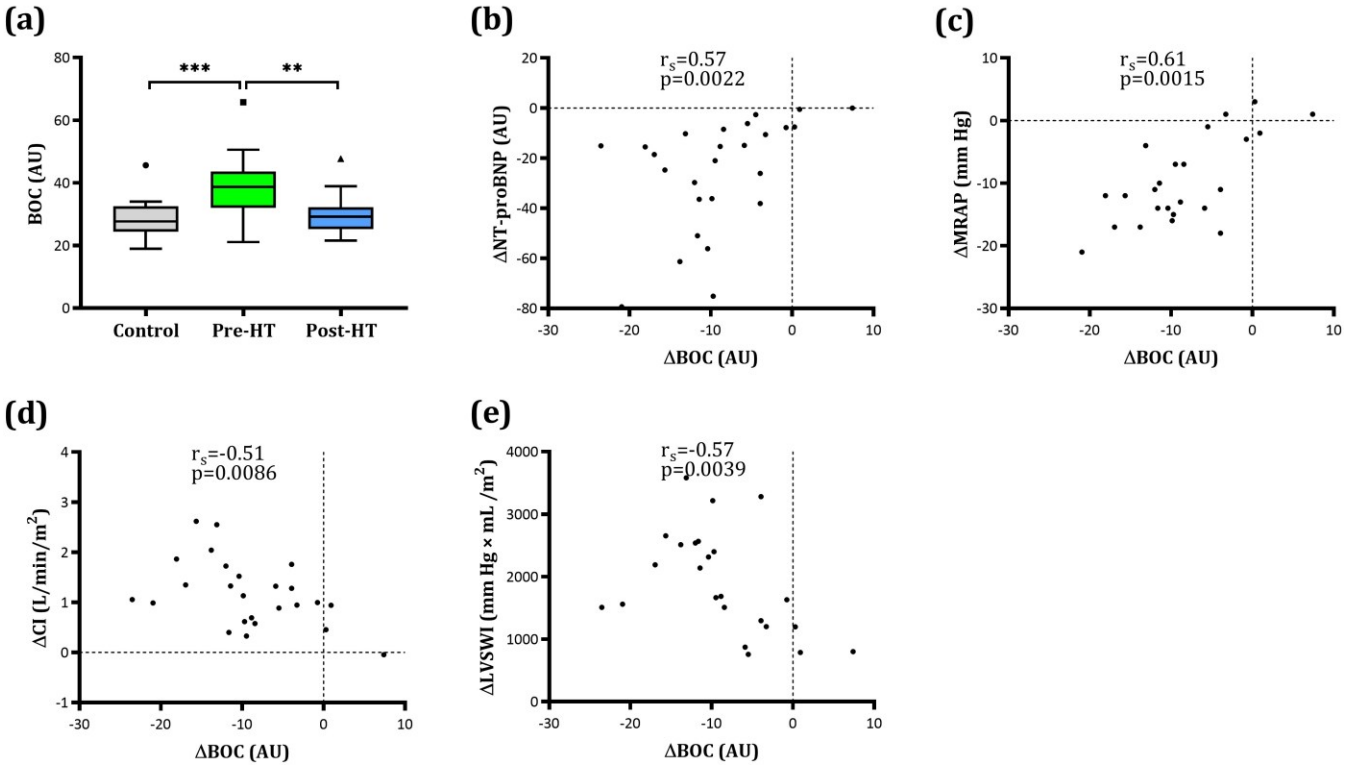

Figure 25.
Plasma levels of **(a)** Brother of CDO (BOC) in controls and in patients with advanced heart failure before and one year after heart transplantation (Pre-HT and Post-HT). Significant correlations **(b – e)** between changes (Post-HT – Pre-HT values, Δ) in plasma levels and haemodynamic parameters. The decrease in BOC levels after heart transplantation correlated with a corresponding decrease in NT-proBNP and MRAP **(b – c)**, as well as an increase in CI and LVSWI **(d – e)**. Tukey's fence was used to define outliers. Abbreviations: AU, arbitrary units, CI, cardiac index, MRAP, mean right atrial pressure, NT-proBNP, N-terminal pro-B-type natriuretic peptide, LVSWI, left ventricular stroke work index, and r_s, Spearman's correlation coefficient. ** $p < 0.001$; *** $p < 0.0001$. False discovery rate < 0.01 in **(a)**, and < 0.1 in **(b – e)**. Adapted from **paper I** under the Creative Commons Attribution license (CC BY).

Paper III

Study population

The median time on the HT waiting list was 98.5 (64 – 168) days. Patients were observed for a median duration of 8.1 (7.5 – 9.2) years during the study follow-up time from 11 November 2011 to 30 May 2022. During follow-up, 6 patients (23.1 %) died (**Figure 26**). Patients' haemodynamics are presented in **Table 7**.

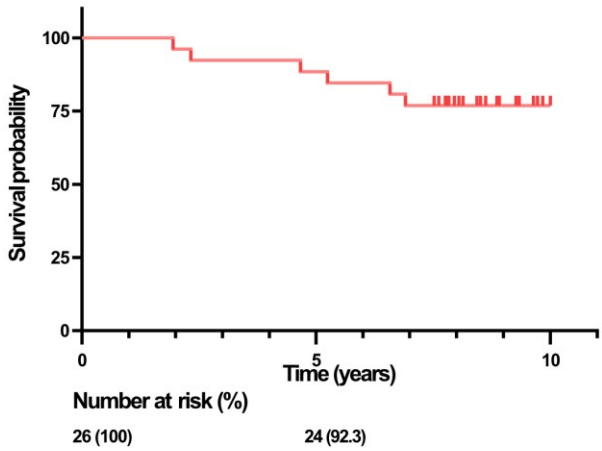

Figure 26.
Survival of patients with advanced heart failure at time of inclusion during the follow-up time between 2011 and 2022. Adapted from **paper III** under the Creative Commons Attribution license (CC BY).

Plasma protein levels and repeated measures correlations

Patients with advanced HF had the most pronounced reductions in plasma VEGF-D and soluble fms-like tyrosine kinase-1 (sFlt-1) levels at four-weeks post-HT ($p < 0.0001$, FDR < 0.01). These levels continued to decrease at six-months ($p < 0.05$) and one-year after HT ($p < 0.05$, **Figure 27**). Over time, plasma VEGF-D and sFlt1 correlated strongest and with the largest number of haemodynamic parameters throughout the first year after HT ($p < 0.01$, **Table 9**).

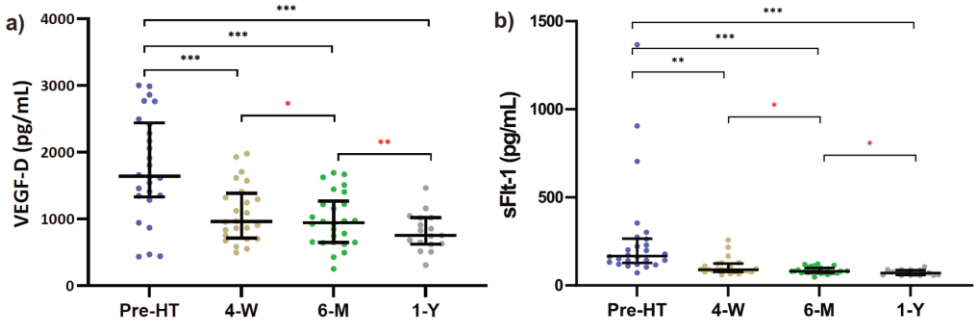

Figure 27.
Absolute plasma concentrations of **(a)** vascular endothelial growth factor D (VEGF-D) and **(b)** soluble fms-like tyrosine kinase-1 (sFlt-1) before- and at multiple follow-ups after heart transplantation (HT). Abbreviations: 4-W indicates four-weeks after HT; 6-M, six-months after HT; and 1-Y, one-year after HT. *** $p < 0.001$; ** p-value < 0.01; * p-value < 0.05. False discovery rate < 0.01 was applied to all comparisons, unless indicated with red asterisk, for which significance was set at $p < 0.05$. Adapted from **paper III** under the Creative Commons Attribution license (CC BY).

Table 9. Repeated measures correlation analyses between plasma levels of VEGF-D and sFlt-1 with haemodynamic parameters at baseline, 4 weeks-, 6-months-, and 1-year after heart transplantation

Parameter	r_{mr} (95% CI_B, p-value)
sFlt-1 (pg/ml) vs:	
mPAP (mmHg)	0.61 (0.51 to 0.76, 2.4×10^{-7})
PAWP (mmHg)	0.66 (0.58 to 0.79, 1.0×10^{-8})
MRAP (mmHg)	0.64 (0.58 to 0.81, 3.9×10^{-8})
CI (liter/min/m^2)	−0.56 (−0.76 to −0.48, 3.5×10^{-6})
PVR (WU)	0.43 (0.33 to 0.72, 0.00062)
PAC (ml/mmHg)	−0.34 (−0.51 to −0.19, 0.008)
VEGF-D (pg/ml) vs:	
mPAP (mmHg)	0.74 (0.58 to 0.82, 7.1×10^{-12})
PAWP (mmHg)	0.75 (0.61 to 0.84, 5.3×10^{-12})
MRAP (mmHg)	0.74 (0.61 to 0.82, 8.0×10^{-12})
CI (liter/min/m^2)	−0.58 (−0.74 to −0.42, 9.3×10^{-7})
PVR (WU)	0.57 (0.43 to 0.76, 1.9×10^{-6})
PAC (ml/mmHg)	−0.39 (−0.59 to −0.24, 0.002)

Abbreviations: CI_B indicates bootstrapped confidence interval; CI, cardiac index; mPAP, mean pulmonary arterial pressure; MRAP, mean right atrial pressure; PAC, pulmonary arterial compliance; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; r_{mr}, repeated measures correlation coefficient; sFlt-1, soluble fms-like tyrosine kinase-1 or soluble VEGF receptor 1 (sVEGFR-1); VEGF-D, vascular endothelial growth factor D; and WU, wood units. All correlations were statistically significant; false discovery rate < 0.01. Adapted from **paper III** under the Creative Commons Attribution license (CC BY).

Paper IV – V

Study population

The demographic and haemodynamic data of the patients are presented in **Table 7**, while the control cohort has been described as abovementioned under **papers I – II**. The event-free survival of LHF-PH (**paper V**) during the study follow-up between 31 October 2011 and 21 August 2020, is described in **Table 10** and **Figure 28**.

Table 10. Survival and event data of heart failure patients in papers IV – V

Variable	LHF-PH (n = 67)	HFpEF-PH (n = 31)	HFrEF-PH (n = 36)
Death, n (%)	25 (37.3)	16 (51.6)	9 (29)
HT during follow-up, n (%)	36 (53.7)	1 (3.2)	35 (92.7)
Events n (%)	53 (79.1)	17 (54.8)	36 (100)
	Median (IQR)	Median (IQR)	Median (IQR)
Overall survival (years)	4.60 (2.87 – 6.77)	3.83 (1.33 – 4.87)	6.20 (4.13 – 7.71)
HT-free survival (years)	0.76 (0.26 – 3.78)	3.78 (1.33 – 4.77)	0.33 (0.19 – 0.73)

Events were defined as death or transplantation (**paper V**). Abbreviations: HFpEF-PH indicates heart failure with preserved ejection fraction and pulmonary hypertension; HFrEF-PH, HF with reduced EF and PH; HT, heart transplanted/transplantation; LHF-PH, left HF with PH, and IQR, interquartile range.

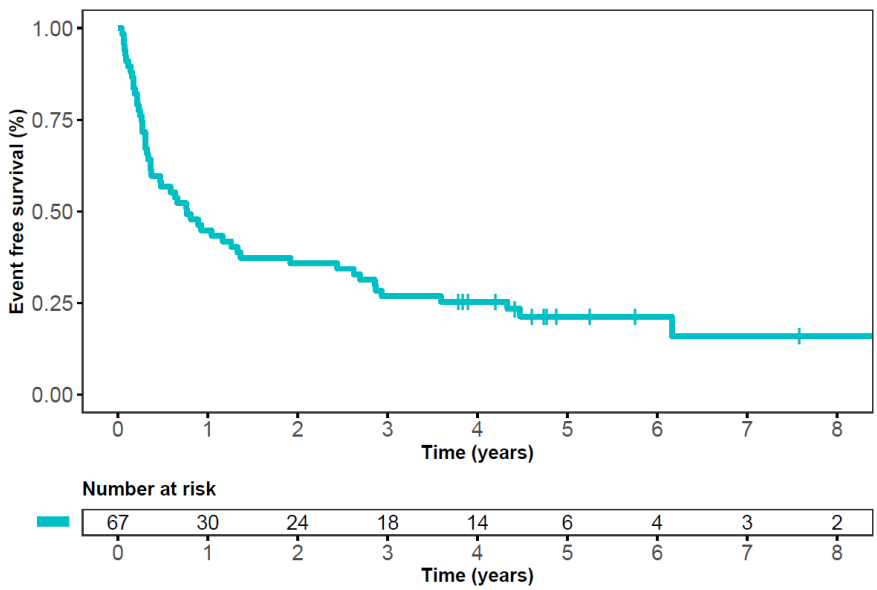

Figure 28.
Transplantation-free survival in patients with left heart failure and pulmonary hypertension (LHF-PH, n = 67) during the study follow-up between 31 October 2011 and 21 August 2020. Adapted from **paper V** under the Creative Commons Attribution license (CC BY).

Diagnostic and prognostic plasma proteins

Of initially included 69 tumour and metabolism related proteins, a total of 36 were eligible for further diagnostic- (**paper IV**) or prognostic (**paper V**) analyses (**Figure 21**).

In **paper IV**, AMBP (protein AMBP or alpha-1-microglobulin/bikunin precursor), LPL (lipoprotein lipase) and glyoxalase I emerged as diagnostic biomarkers. Plasma AMBP and LPL levels were elevated in HFpEF-PH compared to controls ($p < 0.01$), HFrEF-PH ($p < 0.05$) and PAH ($p < 0.001$); whereas plasma levels of glyoxalase I were elevated in both HFpEF-PH and HFrEF-PH compared to controls and PAH ($p < 0.001$), (**Table 11**, FDR < 0.01). Plasma AMBP, LPL, and glyoxalase I, alone, and/or adjusted for age and sex, differentiated HFpEF-PH from PAH in uni- and multivariable logistic regression analyses (**Table 12, Figure 29**). The strongest discrimination between HFpEF-PH and PAH was obtained by combining all three proteins, yielding an AUC of 0.87, with a sensitivity of 87.1% and a specificity of 85.4%, consistent with the optimism-adjusted bootstrap AUC of 0.85 (**Table 12, Figure 29**).

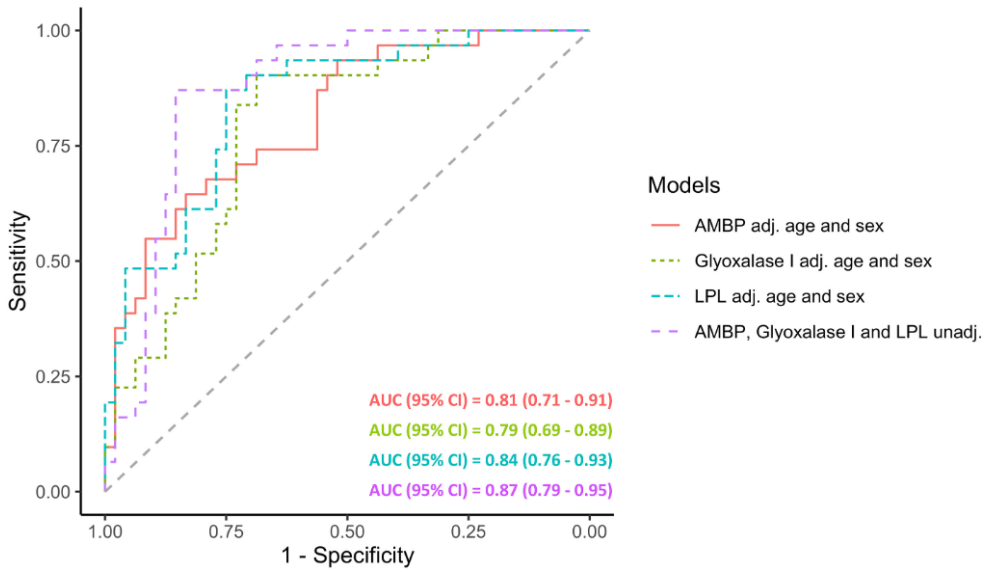

Figure 29.
Receiver operating characteristic curve of protein AMBP or alpha-1-microglobulin/bikunin precursor (AMBP), lipoprotein lipase (LPL) and glyoxalase I logistic regression models in HFpEF-PH differentiation from PAH. Abbreviations: AUC indicates area under the curve; and CI, confidence interval. Adapted from **paper IV** under the Creative Commons Attribution license (CC BY).

Table 11. Plasma levels of key diagnostic and prognostic biomarkers of the study population in papers IV and V

Protein (AU)	Control (n = 20) Median (IQR)	LHF-PH (n = 67) Median (IQR)	HFpEF-PH (n = 31) Median (IQR)	HFrEF-PH (n = 36) Median (IQR)	PAH (n = 48) Median (IQR)
Diagnostic biomarkers					
AMBP	96. (87.5 – 108.1) [a]	111.8 (98.2 – 125.4)	121.72 (104 – 131.7) [c, d]	105.29 (94.4 – 117)	100.52 (91.8 – 112)
Glyoxalase I	83.5 (54.9 – 178.7) [a, b]	237.4 (166.1 – 319.5)	238.38 (166.1 – 316.1) [d]	220 (162.7 – 327.3)	85.8 (56.9 – 256.3)
LPL	987 (925 – 1143) [a]	1140 (851 – 1379)	1216 (1132 – 1465) [c, d]	966 (758 – 1297)	908.1 (752 – 1087)
Prognostic biomarkers					
Endocan	320 (261 – 387) # [a, b]	542 (440 – 702) #	477 (395 – 626) [d]	616 (463 – 789) #	423.04 (343 – 511)
FGF-23	13.9 (11.1 – 16.0) [a, b]	74.3 (35.4 – 289.9)	46.6 (21.6 – 103)	110.1 (44.8 – 566.5)	45 (27.5 – 79.2)
IGF1R	8.57 (7.86 – 9.8) # [a, b]	12.75 (10.84 – 17.65) #	11.99 (10.79 – 14.04) [d]	13.88 (11.26 – 18.15) #	10.38 (8.82 – 12.08)
IGFBP7	12.35 (10.02 – 13.58) [a, b]	23.17 (17.4 – 38.9)	18.42 (15.69 – 29.02) [d]	28.31 (19.64 – 45.1)	15.4 (12.51 – 21.13)
sRAGE	27.05 (25.08 – 29.76) [a, b]	52.93 (42.12 – 66.26)	44.4 (36.37 – 52.93) [c, d]	62.38 (50.89 – 70.01)	38.93 (29.72 – 46.74)

Abbreviations: AU indicates arbitrary units; AMBP, protein AMBP or alpha-1-microglobulin/bikunin precursor; FGF-23, fibroblast growth factor 23; HFpEF-PH, Heart failure with preserved ejection fraction and pulmonary hypertension; HFrEF-PH, HF with reduced EF and PH; IGF1R, insulin-like growth factor 1 receptor; IGFBP7, insulin-like growth factor-binding protein 7; IQR, interquartile range; LHF-PH, left HF with PH; LPL, lipoprotein lipase; PAH, pulmonary arterial hypertension; and sRAGE, soluble receptor for advanced glycation end products.

[a] Significant difference vs HFpEF-PH, $p < 0.014$, FDR < 0.01. [b] Significant difference vs HFrEF-PH, $p < 0.014$, FDR < 0.01. [c] Significant difference vs HFrEF-PH, $p < 0.014$, FDR < 0.01. [d] Significant difference vs PAH, $p < 0.004$, FDR < 0.01. # indicates n – 1.

Table 12. Univariable regression analysis of key diagnostic and prognostic biomarker proteins

Independant variable	Total n (event)	OR or HR	95% CI	P-value
Diagnostic porteins - Univariable logistic regression				
Age (years)	79 (31)	1.058	1.013 – 1.12	0.021
Sex (female)	79 (31)	0.36	0.12 – 1.036	0.061
AMBP (AU)	79 (31)	1.052	1.024 – 1.086	0.00057
Glyoxalase I (AU)	79 (31)	1.0080	1.0038 – 1.013	0.00049
LPL (AU)	79 (31)	1.0031	1.0015 – 1.0050	0.00045
Prognostic porteins - Univariable Cox regression				
Age (years)	67 (53)	0.97	0.96 – 0.99	0.0011
Sex (female)	67 (53)	0.47	0.26 – 0.84	0.011
Atrial fibrillation (yes)	67 (53)	0.52	0.30 – 0.91	0.021
Diabetes mellitus (yes)	64 (52)	0.80	0.41 – 1.56	0.50
Systemic hypertension (yes)	63 (51)	0.37	0.20 – 0.68	0.0013
Endocan (AU)	66 (52)	1.001	1.00 – 1.003	0.010
FGF-23 (AU)	67 (53)	1.001	1.00 – 1.001	0.012
IGF1R (AU)	66 (52)	1.057	1.016 – 1.10	0.0063
IGFBP-7 (AU)	67 (53)	1.011	1.001 – 1.021	0.031
sRAGE (AU)	67 (53)	1.030	1.01 – 1.043	0.0015

In the diagnostic logistic regression analyses, heart failure with preserved ejection fraction and pulmonary hypertension were defined as events, and pulmonary aterial hypertension as non-events. In the Cox regression analyses, heart transplantation or death were defined as events, and survival as non-events. P-values < 0.05 were considered statistically significant. Abbreviations: AU indicates arbitrary units; AMBP, protein AMBP or alpha-1-microglobulin/bikunin precursor; CI, confidence interval; FGF-23, fibroblast growth factor 23; HR, hazard ratio; IGF1R, insulin-like growth factor 1 receptor; IGFBP7, insulin-like growth factor-binding protein 7; LPL, lipoprotein lipase; OR, odds ratio; and sRAGE, soluble receptor for advanced glycation end products. Adapted from **papers IV** and **V** under the Creative Commons Attribution license (CC BY).

In **paper V**, plasma levels of endocan, fibroblast growth factor 23 (FGF-23), insulin-like growth factor 1 receptor (IGF1R), insulin-like growth factor-binding protein 7 (IGFBP7) and soluble receptor for advanced glycation end products (sRAGE) were elevated in HFpEF-PH compared to controls ($p < 0.014$). Except for sRAGE ($p < 0.004$), the levels of the other four proteins did not differ between HFpEF-PH and HFrEF-PH (**Table 11**). Endocan had the largest crude AUC in predicting death or transplantation [0.77 (0.65 – 0.90)], followed by sRAGE [0.75 (0.60 – 0.90)], IGF1R [0.73 (0.59 – 0.87)], FGF-23 [0.72 (0.55 – 0.89)] and IGFBP7 [0.71 (0.55 – 0.87)]. When dichotomized, higher levels of each of the five proteins were associated with worse event-free survival in LHF-PH in the Kaplan-Meier analyses ($p < 0.05$). In the univariable Cox regression analyses, all five proteins retained their prognostic ability ($p < 0.05$). However, after adjustment for age, sex, atrial fibrillation and diabetes mellitus, none of the proteins remained significant predictors of event-free survival in LHF-PH, although plasma sRAGE showed a borderline association ($p = 0.07$, *described in the attached paper V*).

Correlation analyses between plasma protein levels, haemodynamic parameters and NT-proBNP

Among the three diagnostic biomarker proteins, AMBP showed a moderate correlation with PAWP ($r_s = -0.42$, $p = 0.018$) in patients with HFpEF-PH. In patients with LHF-PH, among the univariable prognostic proteins, endocan correlated with the largest number of parameters, followed by FGF-23, IGFBP7, sRAGE and IGF1R ($p < 0.024$, FDR < 0.05, *described in the attached paper V*).

Discussion

HF is a common syndrome in which postcapillary PH frequently develops as a complication, increasing both morbidity and mortality.[147] In advanced cases, HT remains the ultimate treatment.[24, 25, 96] Non-invasive, biomarker-based monitoring of haemodynamic responses in advanced HF before and following HT may have significant implications, including earlier detection of clinical deterioration, timely referral for RHC (re)evaluation and listing for HT, particularly in the stagnant supply of donor grafts. Such biomarkers may also enable more refined disease phenotyping and yield deeper mechanistic insights, ultimately facilitating the development of new therapies.[170]

In line with the rising prevalence of HF, PAH – a rare condition, is increasingly being recognised as a disease of elderly women with comorbidities, exhibiting clinical features closely resembling those of HFpEF-PH, posing clinical challenges.[119, 125, 127, 129-132] Beyond the adverse consequences posed by the diagnostic delay in PAH, misdiagnosis of HFpEF-PH as PAH may consequently occur, potentially leading to detrimental outcomes.[109, 111, 147, 151] Structured evaluation is therefore paramount to enable early identification of HF prior to disease progression to advanced phenotype, as well as to identify PAH amid the complex landscape of dyspnoea. When it is clinically challenging, complementary strategies including novel biomarkers may facilitate the distinction between HFpEF-PH and PAH, thereby reducing diagnostic delays, the risk of misdiagnosis and inappropriate treatment decisions.[24, 25, 137]

Paper I

Among the 28 initially investigated proteins related to tyrosine kinase signalling, VEGF-D and HER4 emerged as the most promising biomarkers, presenting with elevated levels in advanced HF with a subsequent decrease, in line with improved haemodynamics one year after HT. Tyrosine kinase signalling is essential for cardiac development and has a pivotal regulatory role in endothelial function, vascular integrity, angiogenesis, and cardiac remodelling.[180, 181]

Increased intracardiac filling pressures, capillary-stress failure, and extravascular fluid accumulation constitute consequences of myocardial dysfunction in left HF and related PH.[14, 108, 149, 150] VEGF-D is a secreted glycoprotein, most abundantly expressed in the

lungs, and by binding to VEGF receptors 2 and/or 3, VEGF-D regulates a variety of functions including endothelial proliferation, angiogenesis, and cardiac remodelling.[182-185] It also plays a central role in the maintenance of lymphatic vessel system by regulating lymphatic drainage, lymphatic sprouting and tissue fluid homeostasis.[185, 186] Elevated circulating levels of VEGF-D have been reported in lymphangioleiomyomatosis, atrial fibrillation, ischaemic stroke, pre-haemodialysis fluid overload, HF, pulmonary congestion, dyspnoea and PH.[187-193]

Intriguingly, despite elevated intracardiac filling pressures in HF, only mild signs of pulmonary venous congestion and limited pulmonary oedema may be observed.[194, 195] A plausible explanation may be an adaptive rise in lymphatic drainage and flow rate, reported in both animal models and human cases of chronic HF.[196, 197] In patients with HF, circulating VEGF-D was found to be elevated and correlated with increases in PAWP, which is a surrogate for elevated LV end-diastolic pressure. The mechanistic pathways by which lymphatic drainage and angiogenesis occur in HF and PH are yet to be elucidated.[190] The association of ΔVEGF-D with ΔPAWP following HT and reversal of HF in the present study, further support the notion that VEGF-D levels may be related to congestion, and may act to regulate/increase lymphatic drainage in response to HF and pulmonary congestion. Moreover, plasma ΔVEGF-D correlated with other key haemodynamics including ΔmPAP, ΔPVR and ΔPAC, which may suggest that increased VEGF-D levels in HF and/or associated PH may reflect or play a role in pulmonary vasoconstriction and/or vascular remodelling in PH-LHD.

HER4 downstream signalling is crucial for cardiac development, ventricular growth and functional homeostasis of the adult heart.[181] The neuregulin-1/HER2/HER4 axis promotes cardiac repair mechanisms, and has been shown to improve LVEF in both murine and human trials.[181, 198, 199] Intravenous administration of recombinant neuregulin-1 in animal models of ischaemic-, dilated- and viral cardiomyopathies improved cardiac function and survival.[199] Given its potential cardioprotective effects, the efficacy of neuregulin-1 administration is currently being investigated as a therapeutic approach for congestive HF.[198] Our results align with these findings and support that HER4 levels may be related to HF. This view is further reinforced by the correlations between HER4 levels and improved cardiac index and normalised MRAP and NT-proBNP after HT. It remains unknown, however, how the soluble form of HER4, presumably measured in the present study, operates in HF.[200]

Paper II

Of the 48 initially investigated proteins related to tumour signalling, endocan and BOC were most promising, presenting with elevated plasma levels in advanced HF prior to HT, which decreased with a corresponding improvement in invasive haemodynamics one year after HT. A growing body of evidence suggests that common signalling pathways are present between HF, PH and cancer, including neurohormonal

activation, deranged energetics, resistance to apoptosis and inflammation.[40, 166, 168, 201] Intriguingly, it has also been postulated that HF per se may cause cancer, although more studies are needed to support this notion.[166]

Endothelial dysfunction (characterised by an imbalance in the production of vasoactive substances) is a hallmark of the vascular pathology in PH and HF, and its magnitude has been linked with HF severity and functional capacity.[40, 108, 149, 150, 202] Endocan, a dermatan sulphate proteoglycan, is mainly expressed by endothelial cells, and exerts a wide range of biological functions including neovascularisation and cellular adhesion.[203] Endocan has been described as an indicator of endothelial activation and dysfunction, and elevated levels have been reported in sepsis, systemic hypertension, chronic HF with coronary artery disease, cardiogenic shock, atherosclerosis, renal cell carcinoma, and lung cancer.[203-208] In rodent- and in vitro models of PAH, knockdown or inhibition of elevated endocan levels reversed pulmonary vascular remodelling and prevented tumour necrosis factor-alpha induced vascular permeability.[209]

The present study confirms that plasma endocan levels are elevated in advanced HF and decrease after HT. We further support the notion that the increase in plasma endocan may have a role in and/or drive endothelial dysfunction in HF and related PH, as reflected by its correlations with improved pulmonary vascular haemodynamics after HT.

The hedgehog signalling pathway is central in organ development, adult tissue repair and homeostasis, and its dysregulation has been implicated in medulloblastoma and basal cell carcinoma.[209, 210] In a murine model, hedgehog signalling was crucial for coronary artery maintenance, and reduction in its signalling after myocardial infarction increased infarction size and aggravated heart function.[211] BOC functions as a transmembrane co-receptor which enhances hedgehog signalling through a not fully clear mechanism.[210] In murine myocardial ischaemia, intramyocardial gene therapy enhancing the hedgehog signalling, preserved LV function by reducing fibrosis and cardiomyocyte apoptosis.[212]

Herein, we present that plasma BOC is elevated in advanced HF and decreases after HT in response to HT and improved haemodynamics reflecting cardiac function. Although little is known about BOC in HF and PH, we hypothesise that elevated plasma BOC may act as a surrogate of increased activation of hedgehog signalling, which appear to have cardioprotective roles. Accordingly, therapeutic targeting of this signalling pathway may represent a promising strategy for the development of future HF treatments.

Paper III

In the present study, we found that the absolute concentrations of VEGF-D and sFlt-1 or soluble VEGF receptor 1 (sVEGFR-1) in advanced HF, continued to decrease at

multiple time-points throughout the first year after HT in relation improved invasive haemodynamics. The latest ISHLT guidelines recommend that RHC should be done periodically, at least once every three to six months, or more frequently as clinically indicated, for patients on the waitlist for HT.[96] Beyond providing significant prognostic information on patients awaiting HT, invasive haemodynamic assessments are valuable to continuously re-evaluate urgency of transplantation, guide decision for LVAD implantation, optimise medical therapy, and evaluate end-organ injury.[96, 213]

The first year post-HT is the most vulnerable period, during which mortality rates are highest, which is primarily driven by infectious complications and graft failure.[105] Moreover, early symptoms of acute cardiac allograft rejection are often absent or non-specific, which may delay timely recognition until graft dysfunction advances to a potentially fatal stage.[213] These challenges highlight the critical need to enhance monitoring of advanced HF, and of cardiac allograft after HT. In the present paper, plasma VEGF-D declined most markedly at the four-week post-HT assessment. This finding aligns with results from **paper I** and prior discussed studies, in which VEGF-D may primarily reflect decongestion, as it demonstrated the strongest correlations with PAWP and MRAP, which are surrogates for left- and right-ventricular filling pressures, respectively. Interestingly, however, despite normalised haemodynamics already at six-months after HT, plasma VEGF-D continued to decrease while maintaining intraindividual correlations with pulmonary- and cardiac haemodynamic parameters including PAWP. Therefore, whether the continued decrease in VEGF-D despite normalised haemodynamics along with its correlations with PVR and PAC, is attributable to pulmonary vascular remodelling reversal in PH-LHD or to other mechanisms remains to be elucidated. Taken together, our results support the potential clinical use of plasma VEGF-D as a haemodynamic surrogate and monitoring biomarker in advanced HF and throughout the first year after HT.

Likewise, sFlt-1 displayed a plasma level pattern as VEGF-D, and correlated with both pulmonary- and cardiac haemodynamics in advanced HF, and following HT. The soluble form of VEGFR-1, known as sFlt-1, acts as a decoy receptor and has been postulated to negatively regulate angiogenesis by trapping VEGFs.[214] Elevated sFlt-1 inhibits VEGF-mediated endothelial nitric oxide production and induces hypertension. Additionally, elevated sFlt-1 levels have been reported in preeclampsia, myocardial injury, HF, and in different PH aetiologies, potentially implicated in vascular remodelling. [214-218] In line with previous studies and present findings, we support the notion that sFlt-1 may be linked to vascular remodelling, and clinically, may function as a biomarker of haemodynamic surveillance in advanced HF and after HT.

Paper IV

Of the 69 initially investigated proteins related to tumour and metabolism signalling, plasma AMBP, LPL and glyoxalase I emerged as the most promising biomarkers in

distinguishing HFpEF-PH from PAH. The most recent 2022 ESC/ERS PH guidelines and the 7th WSPH proceedings, propose a variety of methods to assist in distinguishing PAH from HFpEF-PH. These include pre-test probabilities with assumptions to the comorbid profiles, passive leg raise testing, and fluid challenging during RHC. However, these methods still lack robust short- and long-term validation and may yield conflicting results.[109, 151]

The clinical difficulty of distinguishing HFpEF-PH from PAH underscores the fundamental complexity of establishing a diagnosis of HFpEF among patients with dyspnoea, especially in cases of compensated disease without overt congestion in whom haemodynamic abnormalities may manifest only during exercise. As a result, H_2FpEF score and HFA-PEFF score algorithm were developed to support the diagnostic evaluation of HFpEF. However, when compared with RHC, these scores still lack diagnostic accuracy (AUCs: 0.71– 0.845), may yield conflicting results, and, in most cases, classify the majority of patients (> 45 %) in the intermediate risk group of having HFpEF, thereby resulting in the need for further diagnostic evaluation and exercise testing.[219, 220] Moreover, in addition to requiring further robust validation against RHC data in the general dyspnoeic population, the scores' utility in distinguishing HFpEF-PH from PAH are yet to be investigated.

In the present study, the combination of plasma AMBP, LPL and glyoxalase I yielded the largest AUC of 0.87 in identifying HFpEF-PH from PAH, and AMBP, which may have direct clinical implications in the future. The present study prompted additional discussions, including an editorial, a letter to the editor, and our subsequent response (**paper VI**, *with the editorial and letter included as supplementary papers*).[221, 222]

The latest 2022 ESC/ERS PH guidelines suggest a class I recommendation for RHC for suspected PH in patients with left heart disease, if it aids in management decisions, compared to the preceding class IIa recommendation in the 2015 ESC/ERS PH guidelines.[109, 110] While invasive haemodynamics using RHC may be paramount in HF and PH management, PH-LHD accounts for 65 – 80% of all cases of PH. These data imply that a systematic and adequate selection process to prioritise patients is essential in relation to available resources, including the use of additional imaging in calculating the probability of PH, but also the use of emerging biomarkers.[110, 147] The multi-marker panel of plasma AMBP, LPL, and glyoxalase I may additionally hold such potential in the future, although this remains to be investigated.

Paper V

In **paper V**, we investigated the plasma tumour- and metabolism-related proteins in predicting prognosis in patients with LHF-PH. Initially, in the univariable analyses, plasma levels of endocan, FGF-23, IGF1R, IGFBP7, and sRAGE predicted death or transplantation, with AUCs > 0.7. Their plasma levels also correlated with multiple haemodynamic parameters of prognostic value in PH-LHD, such as MRAP and

PVR.[151, 223] However, after adjustment in the multivariable Cox-regression analyses, none of the proteins remained significant predictors of death or transplantation.

Previous studies have demonstrated that each of endocan, IGFBP7, FGF-23 and sRAGE predicts poor HF outcomes. Compared to the present paper, these studies were larger and included more events, allowing for adjustment of other covariates, including diabetes mellitus, renal function, and NT-proBNP.[208, 224-227] Moreover, while the ligand IGF1 may serve as a potential predictor of outcomes in HF, there is limited evidence supporting that its receptor, IGF1R, predicts mortality in HF.[228]

The blunted multivariable predictive value of plasma endocan, FGF-23, IGF1R, IGFBP7, and sRAGE, may have been influenced by unadjusted covariates, including diabetes mellitus, medication use, and LVEF. Additionally, the findings may be partly attributed to statistical limitations, such as multicollinearity, non-linear relationships between dependent and independent variables, or low statistical power, the latter reflected by a near-threshold p-value for sRAGE in the multivariable Cox-regression analysis. Taken together, these results remain inconclusive in relation to prior studies, and further research is warranted to validate our findings.

Strengths and limitations

Major strengths of the present studies include the prospectively collected blood samples, the use of highly characterised patients who underwent extensive and invasive haemodynamic assessment at baseline and follow-ups, as well as the use of proximity extension assays to quantify plasma proteins in **papers I, II, IV,** and **V**. Although providing relative plasma concentrations, proximity extension assays offered high sensitivity and specificity analyses, outperforming traditional protein quantification methods.[171] Another strength is the use of an alternative protein quantification method (multiplex sandwich immunoassays) in **paper III**, which provided absolute plasma concentrations and served to validate and strengthen the key findings from **paper I**.

Limitations include the relatively small study population, which may be attributed to the single-centre nature of the thesis papers, although the population is comparable to similar studies which have resulted in larger investigations. The population size may have also contributed to increased likelihood of false negative results and inadequate statistical power to support certain analyses. Moreover, due to the absence of an external validation cohort at the time of analyses, internal bootstrap validation was employed to support some of our conclusions. Despite the application of FDR correction, false positive results may still be present due to the large number of tests performed. Importantly, our results represent associations and do not directly indicate causality. Future studies should address factors influencing plasma protein levels of the potential biomarkers identified, such as diurnal variation, effects on medication use and dosage, as well as comorbidities beyond those adjusted for in the present papers.

Conclusions

Paper I – II

Plasma levels of VEGF-D and HER4, as well as endocan and BOC, may reflect dysregulation in tyrosine kinase- and cancer/tumour- related signalling pathways, respectively. These proteins could provide additional insights into the pathophysiology of advanced HF and/or associated PH. Their clinical utility as potential biomarkers of pulmonary vascular congestion and cardiac function warrants further investigation.

Paper III

Absolute plasma concentrations of VEGF-D and sFlt-1 appear to be promising monitoring biomarkers of pulmonary vascular congestion in advanced HF and haemodynamic surveillance during the first year after HT. Their potential clinical utility as well as involvement in pulmonary vascular congestion and/or remodelling, however, requires further investigation and validation.

Paper IV

Plasma AMBP, LPL and glyoxalase I emerged as promising biomarkers for differentiating HFpEF-PH from PAH. Future studies are encouraged to validate our findings and further elaborate on the biomarkers' potential pathophysiological roles in left HF, HFpEF and related PH.

Paper V

Plasma endocan, FGF-23, IGF1R, IGFBP7, and sRAGE emerged initially as predictors of transplantation-free survival in LHF-PH but were no longer prognostic after multivariable adjustment. This may be ascribed to inadequate statistical power and/or variables that could not be adjusted for, which can be addressed in future studies.

Future perspectives

In line with the expected increase in the prevalence of HF and related PH, healthcare expenditures are likely to rise due to a corresponding increase in hospitalisations and use of advanced therapies.[15, 25, 35, 109] Consequently, optimised strategies for evaluation, management, and treatment are paramount to maximise the efficiency of available resources, minimise diagnostic delays, and improve quality of life and outcomes.[24, 25, 96, 109, 137, 138] Over the past decades, biomarker research has become a subject of intense inquiry, and a multitude of biomarkers are emerging with numerus functional properties, reflecting the unmet need to improve clinical outcomes. Moreover, the emergence of high-throughput methodologies and artificial intelligence in recent years have been nothing short of spectacular, leading to a paradigm shift towards the way of handling and interpreting data. Hopefully, these advances will have great impact and ease the process of biomarker selection and translation to clinical practice.[45, 170]

However, the field of biomarkers in HF and PH-LHD is still in its early stages, and the use of biomarkers tailored to different clinical purposes, reflecting distinct pathophysiological mechanisms beyond natriuretic peptides, is needed.[24, 25, 109] The heterogeneity and the changing landscape in definitions of HF and PH-LHD subtypes over the years underscore the need for more precise phenotyping.[24, 25, 109, 110, 173] Likewise, more continuous haemodynamic monitoring (for example prior to HT, as an integrated component of mechanical circulatory support devices, and in the post-transplant setting) will hopefully be facilitated by improved devices, wirelessly implanted systems, telemedicine, and biomarker-guided surveillance, enabling timely detection of transplant rejection, as well as haemodynamic and clinical deterioration.

The present thesis explored the potential use of circulating biomarkers across the spectrum of HF and PH-LHD, prior to and after HT, for haemodynamic monitoring, prognostication, and differentiation of HFpEF-PH from PAH. Our findings provided potential insights into the dysregulated pathobiological mechanisms involving tyrosine kinase and cancer-/tumour-related signalling pathways in LHF and related PH, generating protein-specific hypotheses for future investigations. Moreover, a central part of the present thesis was to optimise the evaluation of HF and PH to reduce diagnostic delays. This prompted the introduction of a structured evaluation algorithm of dyspnoea, in which these biomarkers can be incorporated in the future to minimise diagnostic delays in HF and PH (**paper VII**). To ensure generalisability, future independent studies are encouraged to validate these findings.

In view of the above, we have recently initiated a project to validate these biomarkers more comprehensively using a larger population, including an external validation cohort from Copenhagen in Denmark. This represents an important first step towards achieving both clinical- and analytical validation, although additional studies will be required to complete this process. Prior to regulatory approval by the European Medicines Agency (EMA) and the FDA, prospective clinical trials evaluating safety, performance, and clinical utility will be essential to support the biomarkers' potential clinical implementation.[229-231]

Acknowledgements

All praise is due to Allah (SWT), without Whom none of this would have been possible.

Göran Rådegran, my main supervisor. Thank you for your engagement, enthusiasm, and support throughout this thesis and my broader academic journey. I look forward to continued collaboration and deeply appreciate your mentorship, even from thousands of meters up on the Haute Route.

Jakob Lundgren, my assistant supervisor. Thank you for your scientific input, valuable discussions, and entertaining presentations. I look forward to many more collaborations in the future.

Abdulla, fellow PhD student. My twin brother and greatest companion. Thank you for being my fiercest rival in the pursuit of knowledge. May the brotherhood we share walk with us through this life and the hereafter.

Mattias Arvidsson, former PhD student and colleague. Thank you for the productive discussions. I enjoyed our mid-day ice cream breaks, which were brief moments that often brought unexpected insights.

Senior colleague, former PhD student, and echocardiography expert, Anna Werther Evaldsson. Thank you for the insightful discussions. I truly look forward to future collaborations, discussing echocardiography, haemodynamics, and statistics.

Senior cardiologist and fellow PhD student, Raluca Jumatate. Your insightful presentations and discussions on grown-up congenital heart diseases and pregnancy in relation to PAH never ceased to amaze me. Your painting skills are nothing short of amazing!

Ahmad Mahmoud, fellow PhD student, friend and colleague. Thank you for the engaging discussions, your kindness, and generosity. Your commitment to running is genuinely inspiring.

Senior colleagues and cardiologists, Associate Professors Arash Mokhtari and Sasha Koul. Thank you for your valuable input during my halfway assessment and for your clinical supervision.

Associate Professor and paediatric cardiologist Karin Tran Lundmark. Thank you for the opportunity to collaborate with you and your former PhD students Christian Westöö and Oscar van der Have. It has been a true privilege to work with you all, and I eagerly look forward continued and future collaborations.

Associate Professor and senior cardiologist Jørn Carlsen. Thank you for welcoming Abdulla and me to the Department of Cardiology at Rigshospitalet in Copenhagen, Denmark. We greatly appreciated your engagement and discussions during data collection. We look forward to continued collaboration.

Lena Lindén and Monica Magnusson. Thank you for offering administrative support throughout my doctoral journey and beyond. Thank you for the entertaining light-hearted conversations. Your support has meant a lot!

My very talented colleagues and researchers Habib Bouzina, Joanna Säleby, Eveline Löfdahl, Hebba Abdul Rahim, Adam Engel Sällberg, Kriss Kania, Daniel Kempe, Sara Helleberg, Rebecca Rylance, Johannes Tödt, Mikael Zhou, Sacharias von Koch, Anton Håkansson, and all other relatives, friends and colleagues for pleasant company and productive discussions.

All brilliant colleagues and researchers at Helsingborg Hospital and Skåne University Hospital in Lund.

The staff at the hemodynamic lab, especially Anneli Ahlqvist, whose work constituted the basis of these studies. Thank you for your incredible contributions to LCPR.

The members of the Swedish Society of Pulmonary Hypertension (SveFPH). Thank you for the engaging discussions.

All participants, including patients and their relatives, for their valuable contributions to the research data.

My very dear parents Alaa and Dhamiaa, your support, enthusiasm, and encouragement have been much appreciated. My siblings Duha, Mohammed A, Abdulla, Noor, and Abdul Wadod, my brothers-in-law Mohammed Q.A and Rabih, as well as my nephews Omar and Ali, whose presence has been a main source of joy. Thank you all for your support, motivating words and inspiring conversations.

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Papers I – VII and supplementary papers I and II

Printed by Media-Tryck, Lund 2026 NORDIC SWAN ECOLABEL 3041 0903

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FACULTY OF MEDICINE

Section for Cardiology
Department of Clinical Sciences Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2026:2
ISBN 978-91-8021-800-9
ISSN 1652-8220